10/574717
IAP15 Rec'd PCT/PTO 23 MAR 2006

Method for the specific rapid detection of beverage-spoiling microorganisms

The invention is related to a method for the specific fast detection of drink-spoiling microorganisms by *in situ*-hybridization. Moreover, the invention is related to specific oligonucleotide probes which are used in the course of the method for detection as well as kits which contain these oligonucleotide probes.

Under the generic clause "non-alcoholic drinks" groups of beverages are summarized like fruit juices, fruit nectars, fruit concentrates, mashed fruits, soft drinks and waters.

Basically non-alcoholic drinks can, due to their diverse/varying composition of nutrients and growth stimulating substances, be classified as potentially endangered by the growth of a large variety of microorganisms.

According to present knowledge mainly yeasts, molds, lactic acid bacteria, acetic acid bacteria, bacilli and alicyclobacilli are found in non-alcoholic drinks and are thus described as "drink-spoiling" microorganisms.

In general contaminations with these microorganisms do not lead to health defects of the consumer but are associated with turbidity, changes of taste and smell within the endproduct and cause high economic losses for the producing industry by image damage based thereon.

Based on the naturally high conccentrations of fruit acids and a corresponding low pH-value (a pH range from 2.5 to 4.5) in fruit juices and fruit nectars only acidophilic or acidotolerant microorganisms (such as lactic acid bacteria, alicyclobacilli, acid tolerant yeast and mold species) can grow and subsequently lead to a deterioration of these beverages.

A measure for restricting spoilage due to microorganisms is carbonisation of beverages. This method is commonly used for the production of soft drinks. By the

addition of CO_2 almost anaerobic conditions are created in the product and only micro-aerophilic, facultatively anaerobic and anaerobic microorganisms (such as lactic acid bacteria, acetic acid bacteria and yeasts) are able to tolerate this environment.

Non-carbonated beverages are in most cases pasteurised in order to assure a long stability and quality of these products. By pasteurisation all vegetative microorganisms should be killed in a manner as comprehensive as possible. However, spores formed by bacilli or alicyclobacilli are not eliminated by this measure. Furthermore, some mold species are able to sustain this process without damage and subsequently create product damages.

A crucial factor for guaranteeing the biological quality of the beverages is the search for the cause of contamination in order to finally eliminate the same. In general, two ways of contamination are distinguished: contaminations are characterised as primary contaminations when microorganisms are introduced into the process by the raw material or by contamination within the process.

Secondary contaminations are those which appear in the filling area after the actual production of the beverage.

The challenge which arises by these different factors for the microbiological quality control, resides in the comprehensive and fast identification of all cells present in the product in order to be able to initiate corresponding counter measures as fast as possible.

Until now conventional detection of drink-spoiling microorganisms is performed by a several days lasting enrichment of the sample in a selective culture medium followed by light microscopy. Furthermore, for the accurate identification of the

drink-spoiling microorganism further physiological tests (like Gram-staining, sugar consumption tests) need to be carried out.

The disadvantages of this solely cultivation-based method are the long duration of the analysis, which cause significant logistic costs in beverage-producing companies. Furthermore, there is the threat of significant image loss for said company, if, after the delivery of products whose microbiological findings had not yet been inequivocally stated, contaminationen are realised and draw-back actions of the spoiled product batches are required.

In the following the drink-spoiling microorganisms and their state of the art detection is described in detail.

Yeasts and molds:

Microorganisms which can survive heat treatment and cause subsequently problems in the beverages are mainly the molds *Byssochlamys fulva* and *B. nivea*, *Neosartorya fischeri* and *Talaromyces flavus* as well as some yeasts. In carbonated drinks mainly the acid-tolerant, fermentative members of yeasts (*Saccharomyces spp.*, *Dekkera spp.* and *Zygosaccharomyces bailii*) are dominating. Besides the threat of product damage based on taste alterations and turbidity caused by these "fermentative yeasts" there is a potential danger of occasional bursts of the filled bottles.

The detection of yeasts and molds is currently performed by cultivation on corresponding culture media (e.g. SSL-bouillon, OFS-medium, malt-dextrose-medium, wort-agar) and needs between 2 and 7 days. A detection on genus or even species level is very time-consuming and is normally not performed.

Lactic acid bacteria

The members of lactic acid bacteria are Gram-positive, non spore-forming, catalase-negative rods and cocci which are characterised by a very high nutrient demand (above all vitamines, amino acids, purines and pyrimidines). As indicated by the name all lactic acid bacteria are able to produce lactic acid as fermentation product.

Due to their anaerobic growth and for anaerobic microorganisms atypical high tolerance and insensitivity against oxygen they are described as aerotolerant anaerobics.

Up until now the genera *Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Carnobacterium, Bifidobacterium, Enterococcus, Pediococcus, Weissella* and *Streptococcus* are referred to as "lactic acid bacteria".

Lactic acid bacteria play an ambivalent role in the food industry. On the one side their presence is wished and indispensable in some processes such as, e.g., the production of sauerkraut. On the other side their presence in beer or fruit juices can lead to a deterioration of the products. The growth of these bacteria is manifested mainly by turbidity, acidification and formation of gas and slime.

In the non-alcoholic drinks industry mainly the bacterial genera *Leuconostoc, Lactococcus, Lactobacillus, Oenococcus, Weissella* and *Pediococcus* are relevant as contaminants. Lactic acid bacteria are detected by a 5 to 7 days incubation at 25°C on MRS agar (pH 5.7).

<u>Acetic acid bacteria</u>

Bacteria of the genera *Acetobacter, Gluconobacter, Gluconoacetobacter and Acidomonas* are described with the trivial name "acetic acid bacteria". Bacteria of these genera are gram-negative, obligate aerobic, oxidase-negative rods whose optimum growth temperature is at 30°C. Acetic acid bacteria are able to grow also at

pH values of 2.2 to 3.0 and, therefore, can produce product damages in beverages having this pH value.

Phylogenetically, bacteria of this genus are members of the *Alphaproteobacteria*.

The product damages mainly goes along with turbidity and alteration of the taste by the formation of acetic acid and gluconic acid. For the detection of acetic acid bacteria mainly ACM-agar (incubation time: 14 days) and DSM-agar (incubation time: 3 to 5 days) have proved themselves.

Bacilli:

Bacilli are Gram-positive aerobic, partly facultatively anaerobic, mostly catalase-positive spore-forming rods. Up until now *Bacillus coagulans* was mainly identified as spoilage microorganism in the non-alcoholic beverage industry.

The detection is performed by plating the sample on dextrose-caseine-peptone agar or yeast extract-peptone dextrose starch agar and subsequent incubation at 55°C (incubation time: 3 days). In order to activate the spores and to achieve a germination of the spores of *B. coagulans*, respectively, a heat treatment of the sample is recommended at 80°C for 10 min. before the actual incubation.

Alicyclobacilli:

Alicyclobacilli are Gram-positive, aerobic, thermophilic and catalase-positive spore-forming rods. Members of this genus produce ? -alicyclic fatty acids as main fatty acids. Up until now *Alicyclobacillus acidoterrestris* was mainly identified in the non-alcoholic beverage industry as spoilage organism. In rare cases also *A. acidocaldarius* and *A. acidiphilus* were identified in spoiled beverages.

The optimum range of the growth temperature for *Alicyclobacillus* spp. is between 26 and 55°C. The pH range where bacteria of this genus can grow, is between 2.2 and 5.8.

The growth of *A. acidoterrestris* leads to spoilage in fruit juices, which is manifested as alteration of the smell and taste due to the formation of guiacol and di-bromophenol. A contamination with this organism proceeds mostly in a non-apparent way, which means that only in rare cases a turbidity is seen in infected beverages.

Alicyclobacilli can be detected by cultivation for several days at 44 – 46°C on orange serum agar, potato dextrose agar, K-agar, YSG-agar or BAM-agar. Furthermore, for the exact confirmation of the finding a set of physiological tests is necessary. In order to activate the spores and to achieve a germination of the spores of *Alicyclobacillus ssp.*, respectively, heat treatment of the sample is recommended at 80°C for 10 min. before the actual incubation.

The routine detection methods for drink-spoiling microorgansims used so far, are very protracted and are partly too inaccurate and thus prevent fast and effective counter measures in order to save the contaminated product. The inaccuracy of the detection arises from a missing differentiation up to genus and/or species level.

As a logical consequence of the difficulties presented by traditional cultivation methods for the detection of drink-spoiling microorganisms, detection methods on the basis of nucleic acids are suitable for the fast, safe and specific identification of spoilage microorganims in non-alcoholic beverages.

In PCR, which is polymerase chain reaction, a characteristic piece of the respective bacterial genome is amplified with specific primers. If the primer finds its target site, a million-fold amplification of a piece of the inherited material occurs. In the following analysis, for example by an agarose gel separating DNA fragments, a

qualitative evaluation can take place. In the most simple case this leads to the conclusion that target sites for the primers used were present in the tested sample. Further conclusions are not possible; these target sites can originate from both a living bacterium and a dead bacterium, or from naked DNA. Since the PCR reaction is positive also in the presence of a dead bacterium or naked DNA, this often leads to false-positive results. A further refinement of this technique is the quantitative PCR which aims at establishing a correlation between the amount of bacteria present and the amount of amplified DNA. Advantages of the PCR are its high specificity, its ease of application and its low expenditure of time. Its main disadvantages are its high susceptibility to contamination and therefore false-positive results, as well as the aforementioned lacking possibility to discriminate between viable and dead cells, and naked DNA, respectively.

A unique approach to combine the specificity of molecularbiological methods such as PCR and the possibility of visualizing bacteria, which is provided by the antibody methods, is the method of fluorescence *in situ* hybridization (FISH; R.I. Amann, W. Ludwig and K.-H. Schleifer, 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiol. Rev. 59, p. 143-169). Using this method bacteria species, genera or groups can be identified and visualized with high specificity.

The FISH technique is based on the fact that in cells of microorganism there are certain molecules which have been mutated only to a small extent in the course of evolution because of their essential function. These are the 16S and the 23S ribosomal ribonucleic acid (rRNA). Both are components of the ribosomes, the sites of protein biosynthesis, and can serve as specific markers on account of their ubiquitous distribution, their size and their structural and functional constancy (Woese, C.R., 1987. Bacterial evolution. Microbiol. Rev. 51, p. 221-271). Based on a comparative sequence analysis, phylogenetic relationships can be established based on these data alone. For this purpose, the sequence data have to be brought into an

alignment. In the alignment, which is based on the knowledge about the secondary structure and tertiary structure of these macromolecules, the homologous positions of the ribosomal nucleic acids are brought in line with each other.

Based on these data, phylogenetic calculations can be made. The use of the most modern computer technology allows to performe even large-scale calculations fast and effectively, as well as to set up large databases which contain the alignment sequences of the 16S, 18S, 23S and 26S rRNA. Due to the fast access to this data material, newly acquired sequences can be phylogenetically analyzed within a short time. These rRNA databases can be used to design species-specific and genus-specific gene probes. Hereby all available rRNA sequences are compared with each other and probes are designed for specific sequence sites, which specifically target a specific species, genus or group of bacteria.

In the FISH (fluorescence *in situ* hybridization) technique, these gene probes which are complementary to a certain region on the ribosomal target sequence, are intoduced into the cell. The gene probes are generally small, 16-20 bases long, single-stranded deoxyribonucleic acid pieces and are directed against a target region which is characteristic for a bacterial species or a bacterial group. If a fluorescencently labeled gene probe finds its target sequence in a cell of a microorganisms, it binds to it and the cells can be detected by means of a fluorescence microscope because of their fluorescence.

The FISH analysis is always performed on a slide, as for the evaluation the bacteria are visualized, i. e. rendered visible, by irradiation with high-energy light. But herein lies one of the disadvantages of the classical FISH analysis: because by definition only comparatively small volumina can be analyzed on a slide, the sensitivity of the method is not satisfying and not sufficient for a reliable analysis.

The present invention thus combines the advantages of the classical FISH analysis with those of cultivation. A comparatively short cultivation step ensures that the bacteria to be detected are present in sufficient numbers before the bacteria are detected using specific FISH.

The practising of the methods described in the present application for the specific detection of drink-spoiling yeasts of the genera *Zygosaccharomyces, Hanseniaspora, Candida, Brettanomyces, Dekkera, Pichia, Saccharomyces* and *Saccharomycodes* in particular the species *Zygosaccharomyces bailii*, *Z. mellis*, *Z. rouxii*, *Z. bisporus*, *Z. fermentati, Z. microellipsoides*, *Hanseniaspora uvarum*, *Candida intermedia*, *C. crusei (Issatchenkia orientalis)*, *C. parapsilosis*, *Brettanomyces bruxellensis*, *B. naardenensis*, *Dekkera anomala*, *Pichia membranaefaciens*, *P. minuta*, *P. anomala*, *Saccharomyces exiguus*, *S. cerevisiae*, *Saccharomycodes ludwigii* or for the specific detection of drink-spoiling molds of the genera *Mucor, Byssochlamys, Neosartorya, Aspergillus and Talaromyces* in particular species of *Mucor racemosus*, *Byssochlamys nivea*, *Neosartorya fischeri*, *Aspergillus fumigatus and A. fischeri*, *Talaromyces flavus*, *T. bacillisporus* and *T. flavus* or for the specific detection of drink-spoiling bacteria of the genera *Lactobacillus, Leuconostoc, Oenococcus, Weissella, Lactococcus, Acetobacter, Gluconobacter, Gluconoacetobacter, Bacillus and Alicyclobacillus*, in particular species of *Lactobacillus collinoides*, *Leuconostoc mesenteroides*, *L. pseudomesenteroides*, *Oenococcus oeni*, *Bacillus coagulans*, *Alicyclobacillus ssp.*, *A. acidoterrestris*, *A. cycloheptanicus* and *A. herbarius* thus comprises the following steps:

- cultivating the drink-spoiling microorganisms present in the sample to be analysed
- fixing the drink-spoiling microorganisms present in the sample
- incubating the fixed drink-spoiling microorganisms with at least one nucleic acid probe and optionally in combination with a competitor probe, in order to achieve hybridization,
- removing or washing off the non-hybridized nucleic acid probe and

- detecting the drink-spoiling microorganisms hybridized to the nucleic acid probe molecules.

Within the present invention "cultivation" is understood to mean the propagation of the microorganisms present in the sample in a suitable cultivation medium.

For the detection of *yeasts and molds* the cultivation may occur, for example, in SSL-bouillon for 24 hours at 25°C. For the detection of lactic acid bacteria the cultivation may occur, for example, in MRS-bouillon for 48 hours at 30°C. For the detection of acetic acid bacteria the cultivation may occur, for example, on DSM-agar for 48 hours at 28°C. For the detection of bacilli, in particular *B. coagulans*, the cultivation may occur, for example, on dextrose-caseine-peptone agar for 48 hours at 55°C. For the detection of alicyclobacilli the cultivation may occur, for example, in BAM-bouillon for 48 hours at 44°C.

In any case, the person skilled in the art can find suitable cultivation methods in the prior art for each microorganism and each group of microorganisms to be analysed, respectively.

Within the present invention "fixing" of the microorganism is understood as a treatment with which the envelope of the microorganism is made permeable for nucleic acid probes. For fixation, usually ethanol is used. If the cell wall cannot be penetrated by the nucleic acid probes despite of using these techniques, the person skilled in the art will know enough further techniques which lead to the same result. These include, for example, methanol, mixtures of alcohols, low percentage paraformaldehyde solution or a diluted formaldehyde solution, enzymatic treatments or the like.

In a particularly preferred embodiment of the method of the present invention an enzymatic step may follow in order to cause complete cell disintegration of the

microoganisms. Enzymes which can accordingly be used for this step, are, for instance, lysozyme, proteinase K, and mutanolysine. The one skilled in the art will know sufficient suitable techniques and will easily find out which means is particularly suitable for cell disintegration of a certain microorganism.

Within the present invention the fixed microorganisms are incubated with fluorescencently labeled nucleic acid probes for the "hybridization". These nucleic acid probes can, after the fixing, penetrate the cell wall and bind to the target sequence in the cell corresponding to the nucleic acid probe. Binding is to be understood as formation of hydrogen bonds between complementary nucleic acid pieces.

In such case the nucleic acid probe can be complementary to a chromosomal or episomal DNA, but can also be complementary to an mRNA or rRNA of the microorganism to be detected. It is advantageous to select a nucleic acid probe which is complementary to a region present in copies of more than 1 in the microorganism to be detected. The sequence to be detected is preferably present in 500-100,000 copies per cell, especially preferred 1,000-50,000 copies. For this reason the sequence of the rRNA is preferably used as a target site, since the ribosomes as sites of protein biosynthesis are present many thousandfold in each active cell.

The nucleic acid probe within the meaning of the invention may be a DNA or RNA probe comprising usually between 12 and 100 nucleotides, preferably between 15 and 50, more between 17 and 25 nucleotides. The selection of the nucleic acid probes is performed taking into consideration whether a complementary sequence is present in the microorganism to be detected. By this selection of a defined sequence, a species of a microorganism, a genus of a microorganism or an entire microorganism group may be detected. In a probe consisting of 15 nucleotides, the sequences should be 100% complementary. In case of oligonucleotides of more than 15 nucleotides, depending on the length of the oligonucleotide, one or more mismatches are allowed.

To increase the specificity of nucleic acid probes competitor probes can be used. Within the present invention competitor probes are understood to mean in particular oligonucleotides which block possibly undesired bindings of the nucleic acid probes and thereby show a higher sequence similarity to the non-target genera and species of microorganisms, respectively, than to the target genera and species of microorganisms, respectively. By using competitor probes the binding of the nucleic acid probe to the nucleic acid sequence of non-target genera or species of microorganisms can be prevented and thus does not lead to false signals. The non-labelled competitor probe is always used in combination with the labelled oligonucleotide probe.

The competitor probe should be complementary to a nucleic acid sequence having high sequence similarity to the nucleic acid sequence of the genera and species of microorganism, respectively, to be detected. In a particularly preferred embodiment the competitor probe is complementary to the rRNA of non-target genera and species of microorganism, respectively.

Within the meaning of the invention the competitor probe is a DNA or RNA sequence usually comprising between 12 and 100 nucleotides, preferably between 15 and 50, particularly preferably between 17 and 25 nucleotides. By selecting a defined sequence, a bacterial species, a bacterial genus or an entire bacterial group may be blocked. A probe consisting of 15 nucleotides should be 100% complementary to the nucleic acid sequence to be blocked. In case of oligonucleotides consisting of more than 15 nucleotides, depending on the length of the oligonucleotide, one or more mismatches are allowed.

Within the methods of the present invention the nucleic acid probe molecules of the present invention have the following lengths and sequences (all nucleic acid probe molecules are noted in 5'–3' direction).

The nucleic acid probe molecules of the present invention are useful for the specific detection of drink-spoiling yeasts of the genera *Zygosaccharomyces, Hanseniaspora, Candida, Brettanomyces, Dekkera, Pichia, Saccharomyces* and *Saccharomycodes* in particular the species *Zygosaccharomyces bailii*, *Z. mellis*, *Z. rouxii*, *Z. bisporus*, *Z. fermentati, Z. microellipsoides*, *Hanseniaspora uvarum*, *Candida intermedia*, *C. crusei (Issatchenkia orientalis)*, *C. parapsilosis*, *Brettanomyces bruxellensis*, *B. naardenensis*, *Dekkera anomala*, *Pichia membranaefaciens*, *P. minuta*, *P. anomala*, *Saccharomyces exiguus*, *S. cerevisiae*, *Saccharomycodes ludwigii* or for the specific detection of drink-spoiling molds of the genera *Mucor, Byssochlamys, Neosartorya, Aspergillus and Talaromyces* in particular species of *Mucor racemosus*, *Byssochlamys nivea*, *Neosartorya fischeri*, *Aspergillus fumigatus and A. fischeri*, *Talaromyces flavus*, *T. bacillisporus* and *T. flavus* or for the specific detection of drink-spoiling bacteria of the genera *Lactobacillus, Leuconostoc, Oenococcus, Weissella, Lactococcus, Acetobacter, Gluconobacter, Gluconoacetobacter, Bacillus and Alicyclobacillus*, in particular species of *Lactobacillus collinoides*, *Leuconostoc mesenteroides*, *L. pseudomesenteroides*, *Oenococcus oeni*, *Bacillus coagulans*, *Alicyclobacillus ssp.*, *A. acidoterrestris*, *A. cycloheptanicus* and *A. herbarius* and are used correspondingly in the detection method according to the invention.

Within the present invention probes that detect different species of microorganims can be used in combination, in order to enable the simultaneous detection of different microoganisms. This leads likewise to an increase of speed of the detection method.

a) Nucleic acid molecules which specifically detect drink-spoiling yeasts:

SEQ ID No. 1: 5'-GTTTGACCAGATTCTCCGCTC

The sequence SEQ ID No. 1 is particularly useful for the detection of microorganisms of the genus *Zygosaccharomyces*.

SEQ ID No. 2:	5′- GTTTGACCAGATTTTCCGCTCT
SEQ ID No. 3:	5′- GTTTGACCAAATTTTCCGCTCT
SEQ ID No. 4:	5′- GTTTGTCCAAATTCTCCGCTCT

The nucleic acid molecules according to SEQ ID No. 2 to SEQ ID No. 4 are used as unlabelled competitor probes for the detection of microorganisms of the genus *Zygosaccharomyces* in combination with the nucleic acid probe according to SEQ ID No. 1 in order to prevent the binding of the labelled nucleic acid probe specific for members of the genus *Zygosaccharomyces* to nucleic acid sequences, which are not specific for members of the genus *Zygosaccharomyces.*

SEQ ID No. 5:	5′- CCCGGTCGAATTAAAACC
SEQ ID No. 6:	5′- GCCCGGTCGAATTAAAAC
SEQ ID No. 7:	5′- GGCCCGGTCGAATTAAAA
SEQ ID No. 8:	5′- AGGCCCGGTCGAATTAAA
SEQ ID No. 9:	5′- AAGGCCCGGTCGAATTAA
SEQ ID No. 10:	5′- ATATTCGAGCGAAACGCC
SEQ ID No. 11:	5′- AAAGATCCGGACCGGCCG
SEQ ID No. 12	5′- GGAAAGATCCGGACCGGC
SEQ ID No. 13	5′- GAAAGATCCGGACCGGCC
SEQ ID No. 14	5′- GATCCGGACCGGCCGACC
SEQ ID No. 15	5′- AGATCCGGACCGGCCGAC
SEQ ID No. 16	5′- AAGATCCGGACCGGCCGA
SEQ ID No. 17	5′- GAAAGGCCCGGTCGAATT
SEQ ID No. 18	5′- AAAGGCCCGGTCGAATTA
SEQ ID No. 19	5′- GGAAAGGCCCGGTCGAAT
SEQ ID No. 20	5′- AGGAAAGGCCCGGTCGAA
SEQ ID No. 21	5′- AAGGAAAGGCCCGGTCGA

The sequences SEQ ID No. 5 to SEQ ID No. 21 are particularly suitable for the detection of *Zygosaccharomyces bailii*.

SEQ ID No. 22: 5′- ATAGCACTGGGATCCTCGCC

The sequence SEQ ID No. 22 is particularly suitable for the detection of *Zygosaccharomyces fermentati*.

SEQ ID No. 23: 5′- CCAGCCCCAAAGTTACCTTC
SEQ ID No. 24: 5′- TCCTTGACGTAAAGTCGCAG

The sequences SEQ ID No. 23 to SEQ ID No. 24 are particularly suitable for the detection of *Zygosaccharomyces microellipsoides*.

SEQ ID No. 25: 5′- GGAAGAAAACCAGTACGC
SEQ ID No. 26: 5′- CCGGTCGGAAGAAAACCA
SEQ ID No. 27: 5′- GAAGAAAACCAGTACGCG
SEQ ID No. 28: 5′- CCCGGTCGGAAGAAAACC
SEQ ID No. 29: 5′- CGGTCGGAAGAAAACCAG
SEQ ID No. 30: 5′- GGTCGGAAGAAAACCAGT
SEQ ID No. 31: 5′- AAGAAAACCAGTACGCGG
SEQ ID No. 32: 5′- GTACGCGGAAAAATCCGG
SEQ ID No. 33: 5′- AGTACGCGGAAAAATCCG
SEQ ID No. 34: 5′- GCGGAAAAATCCGGACCG
SEQ ID No. 35: 5′- CGGAAGAAAACCAGTACG
SEQ ID No. 36: 5′- GCCCGGTCGGAAGAAAAC
SEQ ID No. 37: 5′- CGCGGAAAAATCCGGACC
SEQ ID No. 38: 5′- CAGTACGCGGAAAAATCC
SEQ ID No. 39: 5′- AGAAAACCAGTACGCGGA
SEQ ID No. 40: 5′- GGCCCGGTCGGAAGAAAA

SEQ ID No. 41: 5′- ATAAACACCACCCGATCC
SEQ ID No. 42: 5′- ACGCGGAAAAATCCGGAC
SEQ ID No. 43: 5′- GAGAGGCCCGGTCGGAAG
SEQ ID No. 44: 5′- AGAGGCCCGGTCGGAAGA
SEQ ID No. 45: 5′- GAGGCCCGGTCGGAAGAA
SEQ ID No. 46: 5′- AGGCCCGGTCGGAAGAAA
SEQ ID No. 47: 5′- CCGAGTGGGTCAGTAAAT
SEQ ID No. 48: 5′- CCAGTACGCGGAAAAATC
SEQ ID No. 49: 5′- TAAACACCACCCGATCCC
SEQ ID No. 50: 5′- GGAGAGGCCCGGTCGGAA
SEQ ID No. 51: 5′- GAAAACCAGTACGCGGAA
SEQ ID No. 52: 5′- TACGCGGAAAAATCCGGA
SEQ ID No. 53: 5′- GGCCACAGGGACCCAGGG
SEQ ID No. 54: 5′- TCACCAAGGGCCACAGGG
SEQ ID No. 55: 5′- GGGCCACAGGGACCCAGG
SEQ ID No. 56: 5′- TTCACCAAGGGCCACAGG
SEQ ID No. 57: 5′- ACAGGGACCCAGGGCTAG
SEQ ID No. 58: 5′- AGGGCCACAGGGACCCAG
SEQ ID No. 59: 5′- GTTCACCAAGGGCCACAG
SEQ ID No. 60: 5′- GCCACAGGGACCCAGGGC
SEQ ID No. 61: 5′- CAGGGACCCAGGGCTAGC
SEQ ID No. 62: 5′- AGGGACCCAGGGCTAGCC
SEQ ID No. 63: 5′- ACCAAGGGCCACAGGGAC
SEQ ID No. 64: 5′- CCACAGGGACCCAGGGCT
SEQ ID No. 65: 5′- CACAGGGACCCAGGGCTA
SEQ ID No. 66: 5′- CACCAAGGGCCACAGGGA
SEQ ID No. 67: 5′- GGGACCCAGGGCTAGCCA
SEQ ID No. 68: 5′- AGGAGAGGCCCGGTCGGA
SEQ ID No. 69: 5′- AAGGAGAGGCCCGGTCGG
SEQ ID No. 70: 5′- GAAGGAGAGGCCCGGTCG

SEQ ID No. 71: 5′- AGGGCTAGCCAGAAGGAG
SEQ ID No. 72: 5′- GGGCTAGCCAGAAGGAGA
SEQ ID No. 73: 5′- AGAAGGAGAGGCCCGGTC
SEQ ID No. 74: 5′- CAAGGGCCACAGGGACCC
SEQ ID No. 75: 5′- CCAAGGGCCACAGGGACC

The sequences SEQ ID No. 25 to SEQ ID No. 75 are particularly suitable for the detection of *Zygosaccharomyces mellis*.

SEQ ID No. 76: 5′- GTCGGAAAAACCAGTACG
SEQ ID No. 77: 5′- GCCCGGTCGGAAAAACCA
SEQ ID No. 78: 5′- CCGGTCGGAAAAACCAGT
SEQ ID No. 79: 5′- CCCGGTCGGAAAAACCAG
SEQ ID No. 80: 5′- TCGGAAAAACCAGTACGC
SEQ ID No. 81: 5′- CGGAAAAACCAGTACGCG
SEQ ID No. 82: 5′- GGAAAAACCAGTACGCGG
SEQ ID No. 83: 5′- GTACGCGGAAAAATCCGG
SEQ ID No. 84: 5′- AGTACGCGGAAAAATCCG
SEQ ID No. 85: 5′- GCGGAAAAATCCGGACCG
SEQ ID No. 86: 5′- GGTCGGAAAAACCAGTAC
SEQ ID No. 87: 5′- ACTCCTAGTGGTGCCCTT
SEQ ID No. 88: 5′- GCTCCACTCCTAGTGGTG
SEQ ID No. 89: 5′- CACTCCTAGTGGTGCCCT
SEQ ID No. 90: 5′- CTCCACTCCTAGTGGTGC
SEQ ID No. 91: 5′- TCCACTCCTAGTGGTGCC
SEQ ID No. 92: 5′- CCACTCCTAGTGGTGCCC
SEQ ID No. 93: 5′- GGCTCCACTCCTAGTGGT
SEQ ID No. 94: 5′- AGGCTCCACTCCTAGTGG
SEQ ID No. 95: 5′- GGCCCGGTCGGAAAAACC
SEQ ID No. 96: 5′- GAAAAACCAGTACGCGGA

SEQ ID No. 97:	5′- CGCGGAAAAATCCGGACC
SEQ ID No. 98:	5′- CAGTACGCGGAAAAATCC
SEQ ID No. 99:	5′- CGGTCGGAAAAACCAGTA
SEQ ID No. 100:	5′- AAGGCCCGGTCGGAAAAA
SEQ ID No. 101:	5′- CAGGCTCCACTCCTAGTG
SEQ ID No. 102:	5′- CTCCTAGTGGTGCCCTTC
SEQ ID No. 103:	5′- TCCTAGTGGTGCCCTTCC
SEQ ID No. 104:	5′- GCAGGCTCCACTCCTAGT
SEQ ID No. 105:	5′- AGGCCCGGTCGGAAAAAC
SEQ ID No. 106:	5′- ACGCGGAAAAATCCGGAC
SEQ ID No. 107:	5′- CCAGTACGCGGAAAAATC
SEQ ID No. 108:	5′- CTAGTGGTGCCCTTCCGT
SEQ ID No. 109:	5′- GAAAGGCCCGGTCGGAAA
SEQ ID No. 110:	5′- AAAGGCCCGGTCGGAAAA
SEQ ID No. 111:	5′- TACGCGGAAAAATCCGGA
SEQ ID No. 112:	5′- GGAAAGGCCCGGTCGGAA
SEQ ID No. 113:	5′- ATCTCTTCCGAAAGGTCG
SEQ ID No. 114:	5′- CATCTCTTCCGAAAGGTC
SEQ ID No. 115:	5′- CTCTTCCGAAAGGTCGAG
SEQ ID No. 116:	5′- CTTCCGAAAGGTCGAGAT
SEQ ID No. 117:	5′- TCTCTTCCGAAAGGTCGA
SEQ ID No. 118:	5′- TCTTCCGAAAGGTCGAGA
SEQ ID No. 119:	5′- CCTAGTGGTGCCCTTCCG
SEQ ID No. 120:	5′- TAGTGGTGCCCTTCCGTC
SEQ ID No. 121:	5′- AGTGGTGCCCTTCCGTCA
SEQ ID No. 122:	5′- GCCAAGGTTAGACTCGTT
SEQ ID No. 123:	5′- GGCCAAGGTTAGACTCGT
SEQ ID No. 124:	5′- CCAAGGTTAGACTCGTTG
SEQ ID No. 125:	5′- CAAGGTTAGACTCGTTGG
SEQ ID No. 126:	5′- AAGGTTAGACTCGTTGGC

The sequences SEQ ID No. 76 to SEQ ID No. 126 are particularly suitable for the detection of *Zygosaccharomyces rouxii*.

SEQ ID No. 127: 5′- CTCGCCTCACGGGGTTCTCA

The sequence SEQ ID No. 127 is particularly suitable for the simultanous detection of *Zygosaccharomyces mellis* and *Zygosaccharomyces rouxii*.

SEQ ID No. 128: 5′- GGCCCGGTCGAAATTAAA
SEQ ID No. 129: 5′- AGGCCCGGTCGAAATTAA
SEQ ID No. 130: 5′- AAGGCCCGGTCGAAATTA
SEQ ID No. 131: 5′- AAAGGCCCGGTCGAAATT
SEQ ID No. 132: 5′- GAAAGGCCCGGTCGAAAT
SEQ ID No. 133: 5′- ATATTCGAGCGAAACGCC
SEQ ID No. 134: 5′- GGAAAGGCCCGGTCGAAA
SEQ ID No. 135: 5′- AAAGATCCGGACCGGCCG
SEQ ID No. 136: 5′- GGAAAGATCCGGACCGGC
SEQ ID No. 137: 5′- GAAAGATCCGGACCGGCC
SEQ ID No. 138: 5′- GATCCGGACCGGCCGACC
SEQ ID No. 139: 5′- AGATCCGGACCGGCCGAC
SEQ ID No. 140: 5′- AAGATCCGGACCGGCCGA
SEQ ID No. 141: 5′- AGGAAAGGCCCGGTCGAA
SEQ ID No. 142: 5′- AAGGAAAGGCCCGGTCGA

The sequences SEQ ID No. 128 to SEQ ID No. 142 are particularly suitable for the detection of *Zygosaccharomyces bisporus*.

SEQ ID No. 143: 5′-CGAGCAAAACGCCTGCTTTG
SEQ ID No. 144: 5′-CGCTCTGAAAGAGAGTTGCC

The sequences SEQ ID No. 143 and SEQ ID No. 144 are particularly suitable for the detection of *Hanseniaspora uvarum*.

SEQ ID No. 145: 5′-AGTTGCCCCCTACACTAGAC
SEQ ID No. 146: 5′-GCTTCTCCGTCCCGCGCCG

The sequences SEQ ID No. 145 and SEQ ID No. 146 are particularly suitable for the detection of *Candida intermedia*.

SEQ ID No. 147: 5′- AGATTYTCCGCTCTGAGATGG

The nucleic acid probe molecule according to SEQ ID No. 147 is used as unlabelled competitor probe for the detection of *Candida intermedia* in combination with the oligonucleotide probe according to SEQ ID No. 146, in order to prevent the binding of the labelled oligonucleotide probe specific for *Candida intermedia* to nucleic acid sequences which are not specific for *Candida intermedia*.

SEQ ID No. 148: 5′- CCTGGTTCGCCAAAAAGGC

The sequence SEQ ID No. 148 is particularly suitable for the detection of *Candida parapsilosis*.

SEQ ID No. 149: 5′-GATTCTCGGCCCCATGGG

The sequence SEQ ID No. 149 is particularly suitable for the detection of *Candida crusei* (*Issatchenkia orientalis*).

SEQ ID No. 150: 5′- ACCCTCTACGGCAGCCTGTT

The sequence SEQ ID No. 150 is particularly suitable for the detection of *Dekkera anomala* and *Brettanomyces (Dekkera) bruxellensis.*

SEQ ID No. 151: 5′- GATCGGTCTCCAGCGATTCA

The sequence SEQ ID No. 151 is particularly suitable for the detection of *Brettanomyces (Dekkera) bruxellensis.*

SEQ ID No. 152: 5′- ACCCTCCACGGCGGCCTGTT

The sequence SEQ ID No. 152 is particularly suitable for the detection of *Brettanomyces (Dekkera) naardenensis.*

SEQ ID No. 153: 5′- GATTCTCCGCGCCATGGG

The sequence SEQ ID No. 153 is particularly suitable for the detection of *Pichia membranaefaciens.*

SEQ ID No. 154: 5′- TCATCAGACGGGATTCTCAC

The sequence SEQ ID No. 154 is particularly suitable for the simultanous detection of *Pichia minuta* and *Pichia anomala.*

SEQ ID No. 155: 5′- CTCATCGCACGGGATTCTCACC
SEQ ID No. 156: 5′- CTCGCCACACGGGATTCTCACC

The nucleic acid probe molecules according to SEQ ID No. 155 and SEQ ID No. 156 are used as unlabelled competitor probes for the simultanous detection of *Pichia minuta and Pichia anomala* in combination with the oligonucleotide probe according to SEQ ID No. 154, in order to prevent the binding of the labelled oligonucleotide

probe specific for *Pichia minuta* and *Pichia anomala,* to nucleic acid sequences which are not specific for *Pichia minuta* and *Pichia anomala.*

SEQ ID No. 157: 5′-AGTTGCCCCCTCCTCTAAGC

The sequence SEQ ID No. 157 is particularly suitable for the detection of *Saccharomyces exiguus.*

SEQ ID No. 158: 5′-CTGCCACAAGGACAAATGGT
SEQ ID No. 159: 5′-TGCCCCCTCTTCTAAGCAAAT

The sequences SEQ ID No. 158 and SEQ ID No. 159 are particularly suitable for the detection of *Saccharomyces ludwigii.*

SEQ ID No. 160: 5′-CCCCAAAGTTGCCCTCTC

The sequence SEQ ID No. 160 is particularly suitable for the detection of *Saccharomyces cerevisiae.*

SEQ ID No. 161: 5′-GCCGCCCCAAAGTCGCCCTCTAC
SEQ ID No. 162: 5′-GCCCCAGAGTCGCCTTCTAC

The nucleic acid probe molecules according to SEQ ID No. 161 and SEQ ID No. 162 are used as unlabelled competitor probes for the detection of *Saccharomyces cerevisiae* in combination with the oligonucleotide probe according to SEQ ID No. 160, in order to prevent the binding of the labelled oligonucleotide probe specific for *Saccharomyces cerevisiae,* to nucleic acid sequences which are not specific for *Saccharomyces cerevisiae.*

b) Nucleic acid probe molecules which specifically detect drink-spoiling molds:

SEQ ID No. 163: 5`-AAGACCAGGCCACCTCAT

The sequence SEQ ID No. 163 is particularly suitable for the detection of *Mucor racemosus.*

SEQ ID No. 164: 5`- CATCATAGAACACCGTCC

The sequence SEQ ID No. 164 is particularly suitable for the detection of *Byssochlamys nivea.*

SEQ ID No. 165: 5`- CCTTCCGAAGTCGAGGTTTT

The sequence SEQ ID No. 165 is particularly suitable for the detection of *Neosartorya fischeri.*

SEQ ID No. 166: 5`- GGGAGTGTTGCCAACTC

The sequence SEQ ID No. 166 is particularly suitable for the simultaneous detection of *Aspergillus fumigatus* and *A. fischeri.*

SEQ ID No. 167: 5`- AGCGGTCGTTCGCAACCCT

The sequence SEQ ID No. 167 is particularly suitable for the detection of *Talaromyces flavus.*

SEQ ID No. 168: 5`- CCGAAGTCGGGGTTTTGCGG

The sequence SEQ ID No. 168 is particularly suitable for the simultaneous detection of *Talaromyces bacillisporus* and *T. flavus.*

c) Nucleic acid probe molecules, which specifically detect drink-spoiling lactic acid bacteria

SEQ ID No. 169: 5′- GATAGCCGAAACCACCTTTC
SEQ ID No. 170: 5′- GCCGAAACCACCTTTCAAAC
SEQ ID No. 171: 5′- GTGATAGCCGAAACCACCTT
SEQ ID No. 172: 5′- AGTGATAGCCGAAACCACCT
SEQ ID No. 173: 5′- TTTAACGGGATGCGTTCGAC
SEQ ID No. 174: 5′- AAGTGATAGCCGAAACCACC
SEQ ID No. 175: 5′- GGTTGAATACCGTCAACGTC
SEQ ID No. 176: 5′- GCACAGTATGTCAAGACCTG
SEQ ID No. 177: 5′- CATCCGATGTGCAAGCACTT
SEQ ID No. 178: 5′- TCATCCGATGTGCAAGCACT
SEQ ID No. 179: 5′- CCGATGTGCAAGCACTTCAT
SEQ ID No. 180: 5′- CCACTCATCCGATGTGCAAG
SEQ ID No. 181: 5′- GCCACAGTTCGCCACTCATC
SEQ ID No. 182: 5′- CCTCCGCGTTTGTCACCGGC
SEQ ID No. 183: 5′- ACCAGTTCGCCACAGTTCGC
SEQ ID No. 184: 5′- CACTCATCCGATGTGCAAGC
SEQ ID No. 185: 5′- CCAGTTCGCCACAGTTCGCC
SEQ ID No. 186: 5′- CTCATCCGATGTGCAAGCAC
SEQ ID No. 187: 5′- TCCGATGTGCAAGCACTTCA
SEQ ID No. 188: 5′- CGCCACTCATCCGATGTGCA
SEQ ID No. 189: 5′- CAGTTCGCCACAGTTCGCCA
SEQ ID No. 190: 5′- GCCACTCATCCGATGTGCAA
SEQ ID No. 191: 5′- CGCCACAGTTCGCCACTCAT
SEQ ID No. 192: 5′- ATCCGATGTGCAAGCACTTC
SEQ ID No. 193: 5′- GTTCGCCACAGTTCGCCACT
SEQ ID No. 194: 5′- TCCTCCGCGTTTGTCACCGG

SEQ ID No. 195: 5′- CGCCAGGGTTCATCCTGAGC
SEQ ID No. 196: 5′- AGTTCGCCACAGTTCGCCAC
SEQ ID No. 197: 5′- TCGCCACAGTTCGCCACTCA
SEQ ID No. 198: 5′- TTAACGGGATGCGTTCGACT
SEQ ID No. 199: 5′- TCGCCACTCATCCGATGTGC
SEQ ID No. 200: 5′- CCACAGTTCGCCACTCATCC
SEQ ID No. 201: 5′- GATTTAACGGGATGCGTTCG
SEQ ID No. 202: 5′- TAACGGGATGCGTTCGACTT
SEQ ID No. 203: 5′- AACGGGATGCGTTCGACTTG
SEQ ID No. 204: 5′- CGAAGGTTACCGAACCGACT
SEQ ID No. 205: 5′- CCGAAGGTTACCGAACCGAC
SEQ ID No. 206: 5′- CCCGAAGGTTACCGAACCGA
SEQ ID No. 207: 5′- TTCCTCCGCGTTTGTCACCG
SEQ ID No. 208: 5′- CCGCCAGGGTTCATCCTGAG
SEQ ID No. 209: 5′- TCCTTCCAGAAGTGATAGCC
SEQ ID No. 210: 5′- CACCAGTTCGCCACAGTTCG
SEQ ID No. 211: 5′- ACGGGATGCGTTCGACTTGC
SEQ ID No. 212: 5′- GTCCTTCCAGAAGTGATAGC
SEQ ID No. 213: 5′- GCCAGGGTTCATCCTGAGCC
SEQ ID No. 214: 5′- ACTCATCCGATGTGCAAGCA
SEQ ID No. 215: 5′- ATCATTGCCTTGGTGAACCG
SEQ ID No. 216: 5′- TCCGCGTTTGTCACCGGCAG
SEQ ID No. 217: 5′- TGAACCGTTACTCCACCAAC
SEQ ID No. 218: 5′- GAAGTGATAGCCGAAACCAC
SEQ ID No. 219: 5′- CCGCGTTTGTCACCGGCAGT
SEQ ID No. 220: 5′- TTCGCCACTCATCCGATGTG
SEQ ID No. 221: 5′- CATTTAACGGGATGCGTTCG
SEQ ID No. 222: 5′- CACAGTTCGCCACTCATCCG
SEQ ID No. 223: 5′- TTCGCCACAGTTCGCCACTC
SEQ ID No. 224: 5′- CTCCGCGTTTGTCACCGGCA

SEQ ID No. 225: 5′- ACGCCGCCAGGGTTCATCCT

SEQ ID No. 226: 5′- CCTTCCAGAAGTGATAGCCG

SEQ ID No. 227: 5′- TCATTGCCTTGGTGAACCGT

SEQ ID No. 228: 5′- CACAGTATGTCAAGACCTGG

SEQ ID No. 229: 5′- TTGGTGAACCGTTACTCCAC

SEQ ID No. 230: 5′- CTTGGTGAACCGTTACTCCA

SEQ ID No. 231: 5′- GTGAACCGTTACTCCACCAA

SEQ ID No. 232: 5′- GGCTCCCGAAGGTTACCGAA

SEQ ID No. 233: 5′- GAAGGTTACCGAACCGACTT

SEQ ID No. 234: 5′- TGGCTCCCGAAGGTTACCGA

SEQ ID No. 235: 5′- TAATACGCCGCGGGTCCTTC

SEQ ID No. 236: 5′- GAACCGTTACTCCACCAACT

SEQ ID No. 237: 5′- TACGCCGCGGGTCCTTCCAG

SEQ ID No. 238: 5′- TCACCAGTTCGCCACAGTTC

SEQ ID No. 239: 5′- CCTTGGTGAACCGTTACTCC

SEQ ID No. 240: 5′- CTCACCAGTTCGCCACAGTT

SEQ ID No. 241: 5′- CGCCGCCAGGGTTCATCCTG

SEQ ID No. 242: 5′- CCTTGGTGAACCATTACTCC

SEQ ID No. 243: 5′- TGGTGAACCATTACTCCACC

SEQ ID No. 244: 5′- GCCGCCAGGGTTCATCCTGA

SEQ ID No. 245: 5′- GGTGAACCATTACTCCACCA

SEQ ID No. 246: 5′- CCAGGGTTCATCCTGAGCCA

SEQ ID No. 247: 5′- AATACGCCGCGGGTCCTTCC

SEQ ID No. 248: 5′- CACGCCGCCAGGGTTCATCC

SEQ ID No. 249: 5′- AGTTCGCCACTCATCCGATG

SEQ ID No. 250: 5′- CGGGATGCGTTCGACTTGCA

SEQ ID No. 251: 5′- CATTGCCTTGGTGAACCGTT

SEQ ID No. 252: 5′- GCACGCCGCCAGGGTTCATC

SEQ ID No. 253: 5′- CTTCCTCCGCGTTTGTCACC

SEQ ID No. 254: 5′- TGGTGAACCGTTACTCCACC

SEQ ID No. 255: 5′- CCTTCCTCCGCGTTTGTCAC
SEQ ID No. 256: 5′- ACGCCGCGGGTCCTTCCAGA
SEQ ID No. 257: 5′- GGTGAACCGTTACTCCACCA
SEQ ID No. 258: 5′- GGGTCCTTCCAGAAGTGATA
SEQ ID No. 259: 5′- CTTCCAGAAGTGATAGCCGA
SEQ ID No. 260: 5′- GCCTTGGTGAACCATTACTC
SEQ ID No. 261: 5′- ACAGTTCGCCACTCATCCGA
SEQ ID No. 262: 5′- ACCTTCCTCCGCGTTTGTCA
SEQ ID No. 263: 5′- CGAACCGACTTTGGGTGTTG
SEQ ID No. 264: 5′- GAACCGACTTTGGGTGTTGC
SEQ ID No. 265: 5′- AGGTTACCGAACCGACTTTG
SEQ ID No. 266: 5′- ACCGAACCGACTTTGGGTGT
SEQ ID No. 267: 5′- TTACCGAACCGACTTTGGGT
SEQ ID No. 268: 5′- TACCGAACCGACTTTGGGTG
SEQ ID No. 269: 5′- GTTACCGAACCGACTTTGGG

The sequences SEQ ID No. 169 to SEQ ID No. 269 are particularly suitable for the detection of *Lactobacillus collinoides.*

SEQ ID No. 270: 5′- CCTTTCTGGTATGGTACCGTC
SEQ ID No. 271: 5′- TGCACCGCGGAYCCATCTCT

The sequences SEQ ID No. 270 to SEQ ID No. 271 are particularly suitable for the detection of members of the genus *Leuconostoc.*

SEQ ID No. 272: 5′- AGTTGCAGTCCAGTAAGCCG
SEQ ID No. 273: 5′- GTTGCAGTCCAGTAAGCCGC
SEQ ID No. 274: 5′- CAGTTGCAGTCCAGTAAGCC
SEQ ID No. 275: 5′- TGCAGTCCAGTAAGCCGCCT
SEQ ID No. 276: 5′- TCAGTTGCAGTCCAGTAAGC

SEQ ID No. 277: 5'- TTGCAGTCCAGTAAGCCGCC
SEQ ID No. 278: 5'- GCAGTCCAGTAAGCCGCCTT
SEQ ID No. 279: 5'- GTCAGTTGCAGTCCAGTAAG
SEQ ID No. 280: 5'- CTCTAGGTGACGCCGAAGCG
SEQ ID No. 281: 5'- ATCTCTAGGTGACGCCGAAG
SEQ ID No. 282: 5'- TCTAGGTGACGCCGAAGCGC
SEQ ID No. 283: 5'- TCTCTAGGTGACGCCGAAGC
SEQ ID No. 284: 5'- CCATCTCTAGGTGACGCCGA
SEQ ID No. 285: 5'- CATCTCTAGGTGACGCCGAA
SEQ ID No. 286: 5'- TAGGTGACGCCGAAGCGCCT
SEQ ID No. 287: 5'- CTAGGTGACGCCGAAGCGCC
SEQ ID No. 288: 5'- CTTAGACGGCTCCTTCCTAA
SEQ ID No. 289: 5'- CCTTAGACGGCTCCTTCCTA
SEQ ID No. 290: 5'- ACGTCAGTTGCAGTCCAGTA
SEQ ID No. 291: 5'- CGTCAGTTGCAGTCCAGTAA
SEQ ID No. 292: 5'- ACGCCGAAGCGCCTTTTAAC
SEQ ID No. 293: 5'- GACGCCGAAGCGCCTTTTAA
SEQ ID No. 294: 5'- GCCGAAGCGCCTTTTAACTT
SEQ ID No. 295: 5'- CGCCGAAGCGCCTTTTAACT
SEQ ID No. 296: 5'- GTGACGCCGAAGCGCCTTTT
SEQ ID No. 297: 5'- TGACGCCGAAGCGCCTTTTA
SEQ ID No. 298: 5'- AGACGGCTCCTTCCTAAAAG
SEQ ID No. 299: 5'- ACGGCTCCTTCCTAAAAGGT
SEQ ID No. 300: 5'- GACGGCTCCTTCCTAAAAGG
SEQ ID No. 301: 5'- CCTTCCTAAAAGGTTAGGCC

The sequences SEQ ID No. 272 to SEQ ID No. 301 are particularly suitable for the simultanous detection of *Leuconostoc mesenteroides* and *Leuconostoc pseudomesenteroides*.

SEQ ID No. 302: 5′- GGTGACGCCAAAGCGCCTTT
SEQ ID No. 303: 5′- AGGTGACGCCAAAGCGCCTT
SEQ ID No. 304: 5′- TAGGTGACGCCAAAGCGCCT
SEQ ID No. 305: 5′- CTCTAGGTGACGCCAAAGCG
SEQ ID No. 306: 5′- TCTAGGTGACGCCAAAGCGC
SEQ ID No. 307: 5′- CTAGGTGACGCCAAAGCGCC
SEQ ID No. 308: 5′- ACGCCAAAGCGCCTTTTAAC
SEQ ID No. 309: 5′- CGCCAAAGCGCCTTTTAACT
SEQ ID No. 310: 5′- TGACGCCAAAGCGCCTTTTA
SEQ ID No. 311: 5′- TCTCTAGGTGACGCCAAAGC
SEQ ID No. 312: 5′- GTGACGCCAAAGCGCCTTTT
SEQ ID No. 313: 5′- GACGCCAAAGCGCCTTTTAA
SEQ ID No. 314: 5′- ATCTCTAGGTGACGCCAAAG
SEQ ID No. 315: 5′- CATCTCTAGGTGACGCCAAA
SEQ ID No. 316: 5′- TCCATCTCTAGGTGACGCCA
SEQ ID No. 317: 5′- CCATCTCTAGGTGACGCCAA
SEQ ID No. 318: 5′- CTGCCTTAGACGGCTCCCCC
SEQ ID No. 319: 5′- CCTGCCTTAGACGGCTCCCC
SEQ ID No. 320: 5′- GTGTCATGCGACACTGAGTT
SEQ ID No. 321: 5′- TGTGTCATGCGACACTGAGT
SEQ ID No. 322: 5′- CTTTGTGTCATGCGACACTG
SEQ ID No. 323: 5′- TTGTGTCATGCGACACTGAG
SEQ ID No. 324: 5′- TGCCTTAGACGGCTCCCCCT
SEQ ID No. 325: 5′- AGACGGCTCCCCCTAAAAGG
SEQ ID No. 326: 5′- TAGACGGCTCCCCCTAAAAG
SEQ ID No. 327: 5′- GCCTTAGACGGCTCCCCCTA
SEQ ID No. 328: 5′- GCTCCCCCTAAAAGGTTAGG
SEQ ID No. 329: 5′- GGCTCCCCCTAAAAGGTTAG
SEQ ID No. 330: 5′- CTCCCCCTAAAAGGTTAGGC
SEQ ID No. 331: 5′- TCCCCCTAAAAGGTTAGGCC

SEQ ID No. 332: 5′- CCCTAAAAGGTTAGGCCACC
SEQ ID No. 333: 5′- CCCCTAAAAGGTTAGGCCAC
SEQ ID No. 334: 5′- CGGCTCCCCCTAAAAGGTTA
SEQ ID No. 335: 5′- CCCCCTAAAAGGTTAGGCCA
SEQ ID No. 336: 5′- CTTAGACGGCTCCCCCTAAA
SEQ ID No. 337: 5′- TTAGACGGCTCCCCCTAAAA
SEQ ID No. 338: 5′- GGGTTCGCAACTCGTTGTAT
SEQ ID No. 339: 5′- CCTTAGACGGCTCCCCCTAA
SEQ ID No. 340: 5′- ACGGCTCCCCCTAAAAGGTT
SEQ ID No. 341: 5′- GACGGCTCCCCCTAAAAGGT

The sequences SEQ ID No. 302 to SEQ ID No. 341 are particularly suitable for the detection of *Leuconostoc pseudomesenteroides*.

SEQ ID No. 342: 5′- ACGCCGCAAGACCATCCTCT
SEQ ID No. 343: 5′- CTAATACGCCGCAAGACCAT
SEQ ID No. 344: 5′- TACGCCGCAAGACCATCCTC
SEQ ID No. 345: 5′- GTTACGATCTAGCAAGCCGC
SEQ ID No. 346: 5′- AATACGCCGCAAGACCATCC
SEQ ID No. 347: 5′- CGCCGCAAGACCATCCTCTA
SEQ ID No. 348: 5′- GCTAATACGCCGCAAGACCA
SEQ ID No. 349: 5′- ACCATCCTCTAGCGATCCAA
SEQ ID No. 350: 5′- TAATACGCCGCAAGACCATC
SEQ ID No. 351: 5′- AGCCATCCCTTTCTGGTAAG
SEQ ID No. 352: 5′- ATACGCCGCAAGACCATCCT
SEQ ID No. 353: 5′- AGTTACGATCTAGCAAGCCG
SEQ ID No. 354: 5′- AGCTAATACGCCGCAAGACC
SEQ ID No. 355: 5′- GCCGCAAGACCATCCTCTAG
SEQ ID No. 356: 5′- TTACGATCTAGCAAGCCGCT
SEQ ID No. 357: 5′- GACCATCCTCTAGCGATCCA

SEQ ID No. 358: 5′- TTGCTACGTCACTAGGAGGC
SEQ ID No. 359: 5′- ACGTCACTAGGAGGCGGAAA
SEQ ID No. 360: 5′- TTTGCTACGTCACTAGGAGG
SEQ ID No. 361: 5′- GCCATCCCTTTCTGGTAAGG
SEQ ID No. 362: 5′- TACGTCACTAGGAGGCGGAA
SEQ ID No. 363: 5′- CGTCACTAGGAGGCGGAAAC
SEQ ID No. 364: 5′- AAGACCATCCTCTAGCGATC
SEQ ID No. 365: 5′- GCACGTATTTAGCCATCCCT
SEQ ID No. 366: 5′- CTCTAGCGATCCAAAAGGAC
SEQ ID No. 367: 5′- CCTCTAGCGATCCAAAAGGA
SEQ ID No. 368: 5′- CCATCCTCTAGCGATCCAAA
SEQ ID No. 369: 5′- GGCACGTATTTAGCCATCCC
SEQ ID No. 370: 5′- TACGATCTAGCAAGCCGCTT
SEQ ID No. 371: 5′- CAGTTACGATCTAGCAAGCC
SEQ ID No. 372: 5′- CCGCAAGACCATCCTCTAGC
SEQ ID No. 373: 5′- CCATCCCTTTCTGGTAAGGT
SEQ ID No. 374: 5′- AGACCATCCTCTAGCGATCC
SEQ ID No. 375: 5′- CAAGACCATCCTCTAGCGAT
SEQ ID No. 376: 5′- GCTACGTCACTAGGAGGCGG
SEQ ID No. 377: 5′- TGCTACGTCACTAGGAGGCG
SEQ ID No. 378: 5′- CTACGTCACTAGGAGGCGGA
SEQ ID No. 379: 5′- CCTCAACGTCAGTTACGATC
SEQ ID No. 380: 5′- GTCACTAGGAGGCGGAAACC
SEQ ID No. 381: 5′- TCCTCTAGCGATCCAAAAGG
SEQ ID No. 382: 5′- TGGCACGTATTTAGCCATCC
SEQ ID No. 383: 5′- ACGATCTAGCAAGCCGCTTT
SEQ ID No. 384: 5′- GCCAGTCTCTCAACTCGGCT
SEQ ID No. 385: 5′- AAGCTAATACGCCGCAAGAC
SEQ ID No. 386: 5′- GTTTGCTACGTCACTAGGAG
SEQ ID No. 387: 5′- CGCCACTCTAGTCATTGCCT

SEQ ID No. 388: 5′- GGCCAGCCAGTCTCTCAACT
SEQ ID No. 389: 5′- CAGCCAGTCTCTCAACTCGG
SEQ ID No. 390: 5′- CCCGAAGATCAATTCAGCGG
SEQ ID No. 391: 5′- CCGGCCAGTCTCTCAACTCG
SEQ ID No. 392: 5′- CCAGCCAGTCTCTCAACTCG
SEQ ID No. 393: 5′- TCATTGCCTCACTTCACCCG
SEQ ID No. 394: 5′- GCCAGCCAGTCTCTCAACTC
SEQ ID No. 395: 5′- CACCCGAAGATCAATTCAGC
SEQ ID No. 396: 5′- GTCATTGCCTCACTTCACCC
SEQ ID No. 397: 5′- CATTGCCTCACTTCACCCGA
SEQ ID No. 398: 5′- ATTGCCTCACTTCACCCGAA
SEQ ID No. 399: 5′- CGAAGATCAATTCAGCGGCT
SEQ ID No. 400: 5′- AGTCATTGCCTCACTTCACC
SEQ ID No. 401: 5′- TCGCCACTCTAGTCATTGCC
SEQ ID No. 402: 5′- TTGCCTCACTTCACCCGAAG
SEQ ID No. 403: 5′- CGGCCAGTCTCTCAACTCGG
SEQ ID No. 404: 5′- CTGGCACGTATTTAGCCATC
SEQ ID No. 405: 5′- ACCCGAAGATCAATTCAGCG
SEQ ID No. 406: 5′- TCTAGCGATCCAAAAGGACC
SEQ ID No. 407: 5′- CTAGCGATCCAAAAGGACCT
SEQ ID No. 408: 5′- GCACCCATCGTTTACGGTAT
SEQ ID No. 409: 5′- CACCCATCGTTTACGGTATG
SEQ ID No. 410: 5′- GCCACTCTAGTCATTGCCTC
SEQ ID No. 411: 5′- CGTTTGCTACGTCACTAGGA
SEQ ID No. 412: 5′- GCCTCAACGTCAGTTACGAT
SEQ ID No. 413: 5′- GCCGGCCAGTCTCTCAACTC
SEQ ID No. 414: 5′- TCACTAGGAGGCGGAAACCT
SEQ ID No. 415: 5′- AGCCTCAACGTCAGTTACGA
SEQ ID No. 416: 5′- AGCCAGTCTCTCAACTCGGC
SEQ ID No. 417: 5′- GGCCAGTCTCTCAACTCGGC

SEQ ID No. 418: 5′- CAAGCTAATACGCCGCAAGA
SEQ ID No. 419: 5′- TTCGCCACTCTAGTCATTGC
SEQ ID No. 420: 5′- CCGAAGATCAATTCAGCGGC
SEQ ID No. 421: 5′- CGCAAGACCATCCTCTAGCG
SEQ ID No. 422: 5′- GCAAGACCATCCTCTAGCGA
SEQ ID No. 423: 5′- GCGTTTGCTACGTCACTAGG
SEQ ID No. 424: 5′- CCACTCTAGTCATTGCCTCA
SEQ ID No. 425: 5′- CACTCTAGTCATTGCCTCAC
SEQ ID No. 426: 5′- CCAGTCTCTCAACTCGGCTA
SEQ ID No. 427: 5′- TTACCTTAGGCACCGGCCTC
SEQ ID No. 428: 5′- ACAAGCTAATACGCCGCAAG
SEQ ID No. 429: 5′- TTTACCTTAGGCACCGGCCT
SEQ ID No. 430: 5′- TTTTACCTTAGGCACCGGCC
SEQ ID No. 431: 5′- ATTTTACCTTAGGCACCGGC
SEQ ID No. 432: 5′- GATTTTACCTTAGGCACCGG
SEQ ID No. 433: 5′- CTCACTTCACCCGAAGATCA
SEQ ID No. 434: 5′- ACGCCACCAGCGTTCATCCT
SEQ ID No. 435: 5′- GCCAAGCGACTTTGGGTACT
SEQ ID No. 436: 5′- CGGAAAATTCCCTACTGCAG
SEQ ID No. 437: 5′- CGATCTAGCAAGCCGCTTTC
SEQ ID No. 438: 5′- GGTACCGTCAAGCTGAAAAC
SEQ ID No. 439: 5′- TGCCTCACTTCACCCGAAGA
SEQ ID No. 440: 5′- GGCCGGCCAGTCTCTCAACT
SEQ ID No. 441: 5′- GGTAAGGTACCGTCAAGCTG
SEQ ID No. 442: 5′- GTAAGGTACCGTCAAGCTGA
SEQ ID No. 443: 5′- CCGCAAGACCATCCTCTAGG
SEQ ID No. 444: 5′- ATTTAGCCATCCCTTTCTGG

The sequences SEQ ID No. 342 to SEQ ID No. 444 are particularly suitable for the detection of *Oenococcus oëni*.

SEQ ID No. 445: 5′- AACCCTTCATCACACACG
SEQ ID No. 446: 5′- CGAAACCCTTCATCACAC
SEQ ID No. 447: 5′- ACCCTTCATCACACACGC
SEQ ID No. 448: 5′- TACCGTCACACACTGAAC
SEQ ID No. 449: 5′- AGATACCGTCACACACTG
SEQ ID No. 450: 5′- CACTCAAGGGCGGAAACC
SEQ ID No. 451: 5′- ACCGTCACACACTGAACA
SEQ ID No. 452: 5′- CGTCACACACTGAACAGT
SEQ ID No. 453: 5′- CCGAAACCCTTCATCACA
SEQ ID No. 454: 5′- CCGTCACACACTGAACAG
SEQ ID No. 455: 5′- GATACCGTCACACACTGA
SEQ ID No. 456: 5′- GGTAAGATACCGTCACAC
SEQ ID No. 457: 5′- CCCTTCATCACACACGCG
SEQ ID No. 458: 5′- ACAGTGTTTTACGAGCCG
SEQ ID No. 459: 5′- CAGTGTTTTACGAGCCGA
SEQ ID No. 460: 5′- ACAAAGCGTTCGACTTGC
SEQ ID No. 461: 5′- CGGATAACGCTTGGAACA
SEQ ID No. 462: 5′- AGGGCGGAAACCCTCGAA
SEQ ID No. 463: 5′- GGGCGGAAACCCTCGAAC
SEQ ID No. 464: 5′- GGCGGAAACCCTCGAACA
SEQ ID No. 465: 5′- TGAGGGCTTTCACTTCAG
SEQ ID No. 466: 5′- AGGGCTTTCACTTCAGAC
SEQ ID No. 467: 5′- GAGGGCTTTCACTTCAGA
SEQ ID No. 468: 5′- ACTGCACTCAAGTCATCC
SEQ ID No. 469: 5′- CCGGATAACGCTTGGAAC
SEQ ID No. 470: 5′- TCCGGATAACGCTTGGAA
SEQ ID No. 471: 5′- TATCCCCTGCTAAGAGGT
SEQ ID No. 472: 5′- CCTGCTAAGAGGTAGGTT
SEQ ID No. 473: 5′- CCCTGCTAAGAGGTAGGT

SEQ ID No. 474: 5′- CCCCTGCTAAGAGGTAGG
SEQ ID No. 475: 5′- TCCCCTGCTAAGAGGTAG
SEQ ID No. 476: 5′- ATCCCCTGCTAAGAGGTA
SEQ ID No. 477: 5′- CCGTTCCTTTCTGGTAAG
SEQ ID No. 478: 5′- GCCGTTCCTTTCTGGTAA
SEQ ID No. 479: 5′- AGCCGTTCCTTTCTGGTA
SEQ ID No. 480: 5′- GCACGTATTTAGCCGTTC
SEQ ID No. 481: 5′- CACGTATTTAGCCGTTCC
SEQ ID No. 482: 5′- GGCACGTATTTAGCCGTT
SEQ ID No. 483: 5′- CACTTTCCTCTACTGCAC
SEQ ID No. 484: 5′- CCACTTTCCTCTACTGCA
SEQ ID No. 485: 5′- TCCACTTTCCTCTACTGC
SEQ ID No. 486: 5′- CTTTCCTCTACTGCACTC
SEQ ID No. 487: 5′- TAGCCGTTCCTTTCTGGT
SEQ ID No. 488: 5′- TTAGCCGTTCCTTTCTGG
SEQ ID No. 489: 5′- TTATCCCCTGCTAAGAGG
SEQ ID No. 490: 5′- GTTATCCCCTGCTAAGAG
SEQ ID No. 491: 5′- CCCGTTCGCCACTCTTTG
SEQ ID No. 492: 5′- AGCTGAGGGCTTTCACTT
SEQ ID No. 493: 5′- GAGCTGAGGGCTTTCACT
SEQ ID No. 494: 5′- GCTGAGGGCTTTCACTTC
SEQ ID No. 495: 5′- CTGAGGGCTTTCACTTCA

The sequences SEQ ID No. 445 to SEQ ID No. 495 are particularly suitable for the detection of bacteria of the genus *Weissella*.

SEQ ID No. 496: 5′ CCCGTGTCCCGAAGGAAC
SEQ ID No. 497: 5′ GCACGAGTATGTCAAGAC
SEQ ID No. 498: 5′ GTATCCCGTGTCCCGAAG
SEQ ID No. 499: 5′ TCCCGTGTCCCGAAGGAA

SEQ ID No. 500: 5′ ATCCCGTGTCCCGAAGGA
SEQ ID No. 501: 5′ TATCCCGTGTCCCGAAGG
SEQ ID No. 502: 5′ CTTACCTTAGGAAGCGCC
SEQ ID No. 503: 5′ TTACCTTAGGAAGCGCCC
SEQ ID No. 504: 5′ CCTGTATCCCGTGTCCCG
SEQ ID No. 505: 5′ CCACCTGTATCCCGTGTC
SEQ ID No. 506: 5′ CACCTGTATCCCGTGTCC
SEQ ID No. 507: 5′ ACCTGTATCCCGTGTCCC
SEQ ID No. 508: 5′ CTGTATCCCGTGTCCCGA
SEQ ID No. 509: 5′ TGTATCCCGTGTCCCGAA
SEQ ID No. 510: 5′ CACGAGTATGTCAAGACC
SEQ ID No. 511: 5′ CGGTCTTACCTTAGGAAG
SEQ ID No. 512: 5′ TAGGAAGCGCCCTCCTTG
SEQ ID No. 513: 5′ AGGAAGCGCCCTCCTTGC
SEQ ID No. 514: 5′ TTAGGAAGCGCCCTCCTT
SEQ ID No. 515: 5′ CTTAGGAAGCGCCCTCCT
SEQ ID No. 516: 5′ CCTTAGGAAGCGCCCTCC
SEQ ID No. 517: 5′ ACCTTAGGAAGCGCCCTC
SEQ ID No. 518: 5′ TGCACACAATGGTTGAGC
SEQ ID No. 519: 5′ TACCTTAGGAAGCGCCCT
SEQ ID No. 520: 5′ ACCACCTGTATCCCGTGT
SEQ ID No. 521: 5′ GCACCACCTGTATCCCGT
SEQ ID No. 522: 5′ CACCACCTGTATCCCGTG
SEQ ID No. 523: 5′ GCGGTTAGGCAACCTACT
SEQ ID No. 524: 5′ TGCGGTTAGGCAACCTAC
SEQ ID No. 525: 5′ TTGCGGTTAGGCAACCTA
SEQ ID No. 526: 5′ GGTCTTACCTTAGGAAGC
SEQ ID No. 527: 5′ GCTAATACAACGCGGGAT
SEQ ID No. 528: 5′ CTAATACAACGCGGGATC
SEQ ID No. 529: 5′ ATACAACGCGGGATCATC

SEQ ID No. 530: 5′ CGGTTAGGCAACCTACTT
SEQ ID No. 531: 5′ TGCACCACCTGTATCCCG
SEQ ID No. 532: 5′ GAAGCGCCCTCCTTGCGG
SEQ ID No. 533: 5′ GGAAGCGCCCTCCTTGCG
SEQ ID No. 534: 5′ CGTCCCTTTCTGGTTAGA
SEQ ID No. 535: 5′ AGCTAATACAACGCGGGA
SEQ ID No. 536: 5′ TAGCTAATACAACGCGGG
SEQ ID No. 537: 5′ CTAGCTAATACAACGCGG
SEQ ID No. 538: 5′ GGCTATGTATCATCGCCT
SEQ ID No. 539: 5′ GAGCCACTGCCTTTTACA
SEQ ID No. 540: 5′ GTCGGCTATGTATCATCG
SEQ ID No. 541: 5′ GGTCGGCTATGTATCATC
SEQ ID No. 542: 5′ CAGGTCGGCTATGTATCA
SEQ ID No. 543: 5′ CGGCTATGTATCATCGCC
SEQ ID No. 544: 5′ TCGGCTATGTATCATCGC
SEQ ID No. 545: 5′ GTCTTACCTTAGGAAGCG
SEQ ID No. 546: 5′ TCTTACCTTAGGAAGCGC

The sequences SEQ ID No. 496 to SEQ ID No. 546 are particularly suitable for the detection of bacteria of the genus *Lactococcus*.

d) Nucleic acid molecules, which specifically detect drink-spoiling acetic acid bacteria:

SEQ ID No. 547: 5′- GTACAAACCGCCTACACGCC
SEQ ID No. 548: 5′- TGTACAAACCGCCTACACGC
SEQ ID No. 549: 5′- GATCAGCACGATGTCGCCAT
SEQ ID No. 550: 5′- CTGTACAAACCGCCTACACG
SEQ ID No. 551: 5′- GAGATCAGCACGATGTCGCC
SEQ ID No. 552: 5′- AGATCAGCACGATGTCGCCA

SEQ ID No. 553: 5′- ATCAGCACGATGTCGCCATC
SEQ ID No. 554: 5′- TCAGCACGATGTCGCCATCT
SEQ ID No. 555: 5′- ACTGTACAAACCGCCTACAC
SEQ ID No. 556: 5′- CCGCCACTAAGGCCGAAACC
SEQ ID No. 557: 5′- CAGCACGATGTCGCCATCTA
SEQ ID No. 558: 5′- TACAAACCGCCTACACGCCC
SEQ ID No. 559: 5′- AGCACGATGTCGCCATCTAG
SEQ ID No. 560: 5′- CGGCTTTTAGAGATCAGCAC
SEQ ID No. 561: 5′- TCCGCCACTAAGGCCGAAAC
SEQ ID No. 562: 5′- GACTGTACAAACCGCCTACA
SEQ ID No. 563: 5′- GTCCGCCACTAAGGCCGAAA
SEQ ID No. 564: 5′- GGGGATTTCACATCTGACTG
SEQ ID No. 565: 5′- CATACAAGCCCTGGTAAGGT
SEQ ID No. 566: 5′- ACAAGCCCTGGTAAGGTTCT
SEQ ID No. 567: 5′- ACAAACCGCCTACACGCCCT
SEQ ID No. 568: 5′- CTGACTGTACAAACCGCCTA
SEQ ID No. 569: 5′- TGACTGTACAAACCGCCTAC
SEQ ID No. 570: 5′- ACGATGTCGCCATCTAGCTT
SEQ ID No. 571: 5′- CACGATGTCGCCATCTAGCT
SEQ ID No. 572: 5′- CGATGTCGCCATCTAGCTTC
SEQ ID No. 573: 5′- GCACGATGTCGCCATCTAGC
SEQ ID No. 574: 5′- GATGTCGCCATCTAGCTTCC
SEQ ID No. 575: 5′- ATGTCGCCATCTAGCTTCCC
SEQ ID No. 576: 5′- TGTCGCCATCTAGCTTCCCA
SEQ ID No. 577: 5′- GCCATCTAGCTTCCCACTGT
SEQ ID No. 578: 5′- TCGCCATCTAGCTTCCCACT
SEQ ID No. 579: 5′- CGCCATCTAGCTTCCCACTG
SEQ ID No. 580: 5′- GTCGCCATCTAGCTTCCCAC
SEQ ID No. 581: 5′- TACAAGCCCTGGTAAGGTTC
SEQ ID No. 582: 5′- GCCACTAAGGCCGAAACCTT

SEQ ID No. 583: 5′- ACTAAGGCCGAAACCTTCGT
SEQ ID No. 584: 5′- CTAAGGCCGAAACCTTCGTG
SEQ ID No. 585: 5′- CACTAAGGCCGAAACCTTCG
SEQ ID No. 586: 5′- AAGGCCGAAACCTTCGTGCG
SEQ ID No. 587: 5′- CCACTAAGGCCGAAACCTTC
SEQ ID No. 588: 5′- TAAGGCCGAAACCTTCGTGC
SEQ ID No. 589: 5′- AGGCCGAAACCTTCGTGCGA
SEQ ID No. 590: 5′- TCTGACTGTACAAACCGCCT
SEQ ID No. 591: 5′- CATCTGACTGTACAAACCGC
SEQ ID No. 592: 5′- ATCTGACTGTACAAACCGCC
SEQ ID No. 593: 5′- CTTCGTGCGACTTGCATGTG
SEQ ID No. 594: 5′- CCTTCGTGCGACTTGCATGT
SEQ ID No. 595: 5′- CTCTCTAGAGTGCCCACCCA
SEQ ID No. 596: 5′- TCTCTAGAGTGCCCACCCAA
SEQ ID No. 597: 5′- ACGTATCAAATGCAGCTCCC
SEQ ID No. 598: 5′- CGTATCAAATGCAGCTCCCA
SEQ ID No. 599: 5′- CGCCACTAAGGCCGAAACCT
SEQ ID No. 600: 5′- CCGAAACCTTCGTGCGACTT
SEQ ID No. 601: 5′- GCCGAAACCTTCGTGCGACT
SEQ ID No. 602: 5′- AACCTTCGTGCGACTTGCAT
SEQ ID No. 603: 5′- CGAAACCTTCGTGCGACTTG
SEQ ID No. 604: 5′- ACCTTCGTGCGACTTGCATG
SEQ ID No. 605: 5′- GAAACCTTCGTGCGACTTGC
SEQ ID No. 606: 5′- GGCCGAAACCTTCGTGCGAC
SEQ ID No. 607: 5′- AAACCTTCGTGCGACTTGCA
SEQ ID No. 608: 5′- CACGTATCAAATGCAGCTCC

The sequences SEQ ID No. 547 to SEQ ID No. 608 are particularly suitable for the simultanous detection of bacteria of the genera *Acetobacter* and *Gluconobacter*.

SEQ ID No. 609: 5′- GCTCACCGGCTTAAGGTCAA
SEQ ID No. 610: 5′- CGCTCACCGGCTTAAGGTCA
SEQ ID No. 611: 5′- TCGCTCACCGGCTTAAGGTC
SEQ ID No. 612: 5′- CTCACCGGCTTAAGGTCAAA
SEQ ID No. 613: 5′- CCCGACCGTGGTCGGCTGCG
SEQ ID No. 614: 5′- GCTCACCGGCTTAAGGTCAA
SEQ ID No. 615: 5′- CGCTCACCGGCTTAAGGTCA
SEQ ID No. 616: 5′- TCGCTCACCGGCTTAAGGTC
SEQ ID No. 617: 5′- CTCACCGGCTTAAGGTCAAA
SEQ ID No. 618: 5′- CCCGACCGTGGTCGGCTGCG
SEQ ID No. 619: 5′- TCACCGGCTTAAGGTCAAAC
SEQ ID No. 620: 5′- CAACCCTCTCTCACACTCTA
SEQ ID No. 621: 5′- ACAACCCTCTCTCACACTCT
SEQ ID No. 622: 5′- CCACAACCCTCTCTCACACT
SEQ ID No. 623: 5′- AACCCTCTCTCACACTCTAG
SEQ ID No. 624: 5′- CACAACCCTCTCTCACACTC
SEQ ID No. 625: 5′- TCCACAACCCTCTCTCACAC
SEQ ID No. 626: 5′- TTCCACAACCCTCTCTCACA
SEQ ID No. 627: 5′- ACCCTCTCTCACACTCTAGT
SEQ ID No. 628: 5′- GAGCCAGGTTGCCGCCTTCG
SEQ ID No. 629: 5′- AGGTCAAACCAACTCCCATG
SEQ ID No. 630: 5′- ATGAGCCAGGTTGCCGCCTT
SEQ ID No. 631: 5′- TGAGCCAGGTTGCCGCCTTC
SEQ ID No. 632: 5′- AGGCTCCTCCACAGGCGACT
SEQ ID No. 633: 5′- CAGGCTCCTCCACAGGCGAC
SEQ ID No. 634: 5′- GCAGGCTCCTCCACAGGCGA
SEQ ID No. 635: 5′- TTCGCTCACCGGCTTAAGGT
SEQ ID No. 636: 5′- GTTCGCTCACCGGCTTAAGG
SEQ ID No. 637: 5′- GGTTCGCTCACCGGCTTAAG
SEQ ID No. 638: 5′- ATTCCACAACCCTCTCTCAC

SEQ ID No. 639:	5′- TGACCCGACCGTGGTCGGCT
SEQ ID No. 640:	5′- CCCTCTCTCACACTCTAGTC
SEQ ID No. 641:	5′- GAATTCCACAACCCTCTCTC
SEQ ID No. 642:	5′- AGCCAGGTTGCCGCCTTCGC
SEQ ID No. 643:	5′- GCCAGGTTGCCGCCTTCGCC
SEQ ID No. 644:	5′- GGAATTCCACAACCCTCTCT
SEQ ID No. 645:	5′- GGGAATTCCACAACCCTCTC
SEQ ID No. 646:	5′- AACGCAGGCTCCTCCACAGG
SEQ ID No. 647:	5′- CGGCTTAAGGTCAAACCAAC
SEQ ID No. 648:	5′- CCGGCTTAAGGTCAAACCAA
SEQ ID No. 649:	5′- CACCGGCTTAAGGTCAAACC
SEQ ID No. 650:	5′- ACCGGCTTAAGGTCAAACCA
SEQ ID No. 651:	5′- ACCCAACATCCAGCACACAT
SEQ ID No. 652:	5′- TCGCTGACCCGACCGTGGTC
SEQ ID No. 653:	5′- CGCTGACCCGACCGTGGTCG
SEQ ID No. 654:	5′- GACCCGACCGTGGTCGGCTG
SEQ ID No. 655:	5′- GCTGACCCGACCGTGGTCGG
SEQ ID No. 656:	5′- CTGACCCGACCGTGGTCGGC
SEQ ID No. 657:	5′- CAGGCGACTTGCGCCTTTGA
SEQ ID No. 658:	5′- TCATGCGGTATTAGCTCCAG
SEQ ID No. 659:	5′- ACTAGCTAATCGAACGCAGG
SEQ ID No. 660:	5′- CATGCGGTATTAGCTCCAGT
SEQ ID No. 661:	5′- CGCAGGCTCCTCCACAGGCG
SEQ ID No. 662:	5′- ACGCAGGCTCCTCCACAGGC
SEQ ID No. 663:	5′- CTCAGGTGTCATGCGGTATT
SEQ ID No. 664:	5′- CGCCTTTGACCCTCAGGTGT
SEQ ID No. 665:	5′- ACCCTCAGGTGTCATGCGGT
SEQ ID No. 666:	5′- CCTCAGGTGTCATGCGGTAT
SEQ ID No. 667:	5′- TTTGACCCTCAGGTGTCATG
SEQ ID No. 668:	5′- GACCCTCAGGTGTCATGCGG

SEQ ID No. 669: 5′- TGACCCTCAGGTGTCATGCG
SEQ ID No. 670: 5′- GCCTTTGACCCTCAGGTGTC
SEQ ID No. 671: 5′- TTGACCCTCAGGTGTCATGC
SEQ ID No. 672: 5′- CCCTCAGGTGTCATGCGGTA
SEQ ID No. 673: 5′- CCTTTGACCCTCAGGTGTCA
SEQ ID No. 674: 5′- CTTTGACCCTCAGGTGTCAT
SEQ ID No. 675: 5′- AGTTATCCCCCACCCATGGA
SEQ ID No. 676: 5′- CCAGCTATCGATCATCGCCT
SEQ ID No. 677: 5′- ACCAGCTATCGATCATCGCC
SEQ ID No. 678: 5′- CAGCTATCGATCATCGCCTT
SEQ ID No. 679: 5′- AGCTATCGATCATCGCCTTG
SEQ ID No. 680: 5′- GCTATCGATCATCGCCTTGG
SEQ ID No. 681: 5′- CTATCGATCATCGCCTTGGT
SEQ ID No. 682: 5′- TTCGTGCGACTTGCATGTGT
SEQ ID No. 683: 5′- TCGATCATCGCCTTGGTAGG
SEQ ID No. 684: 5′- ATCGATCATCGCCTTGGTAG
SEQ ID No. 685: 5′- CACAGGCGACTTGCGCCTTT
SEQ ID No. 686: 5′- CCACAGGCGACTTGCGCCTT
SEQ ID No. 687: 5′- TCCACAGGCGACTTGCGCCT
SEQ ID No. 688: 5′- TCCTCCACAGGCGACTTGCG
SEQ ID No. 689: 5′- CCTCCACAGGCGACTTGCGC
SEQ ID No. 690: 5′- CTCCACAGGCGACTTGCGCC
SEQ ID No. 691: 5′- ACAGGCGACTTGCGCCTTTG
SEQ ID No. 692: 5′- GCTCACCGGCTTAAGGTCAA
SEQ ID No. 693: 5′- CGCTCACCGGCTTAAGGTCA
SEQ ID No. 694: 5′- TCGCTCACCGGCTTAAGGTC
SEQ ID No. 695: 5′- CTCACCGGCTTAAGGTCAAA
SEQ ID No. 696: 5′- CCCGACCGTGGTCGGCTGCG
SEQ ID No. 697: 5′- TCACCGGCTTAAGGTCAAAC
SEQ ID No. 698: 5′- CAACCCTCTCTCACACTCTA

SEQ ID No. 699:	5′- ACAACCCTCTCTCACACTCT
SEQ ID No. 700:	5′- CCACAACCCTCTCTCACACT
SEQ ID No. 701:	5′- AACCCTCTCTCACACTCTAG
SEQ ID No. 702:	5′- CACAACCCTCTCTCACACTC
SEQ ID No. 703:	5′- TCCACAACCCTCTCTCACAC
SEQ ID No. 704:	5′- TTCCACAACCCTCTCTCACA
SEQ ID No. 705:	5′- ACCCTCTCTCACACTCTAGT
SEQ ID No. 706:	5′- GAGCCAGGTTGCCGCCTTCG
SEQ ID No. 707:	5′- AGGTCAAACCAACTCCCATG
SEQ ID No. 708:	5′- ATGAGCCAGGTTGCCGCCTT
SEQ ID No. 709:	5′- TGAGCCAGGTTGCCGCCTTC
SEQ ID No. 710:	5′- AGGCTCCTCCACAGGCGACT
SEQ ID No. 711:	5′- CAGGCTCCTCCACAGGCGAC
SEQ ID No. 712:	5′- GCAGGCTCCTCCACAGGCGA
SEQ ID No. 713:	5′- TTCGCTCACCGGCTTAAGGT
SEQ ID No. 714:	5′- GTTCGCTCACCGGCTTAAGG
SEQ ID No. 715:	5′- GGTTCGCTCACCGGCTTAAG
SEQ ID No. 716:	5′- ATTCCACAACCCTCTCTCAC
SEQ ID No. 717:	5′- TGACCCGACCGTGGTCGGCT
SEQ ID No. 718:	5′- CCCTCTCTCACACTCTAGTC
SEQ ID No. 719:	5′- GAATTCCACAACCCTCTCTC
SEQ ID No. 720:	5′- AGCCAGGTTGCCGCCTTCGC
SEQ ID No. 721:	5′- GCCAGGTTGCCGCCTTCGCC
SEQ ID No. 722:	5′- GGAATTCCACAACCCTCTCT
SEQ ID No. 723:	5′- GGGAATTCCACAACCCTCTC
SEQ ID No. 724:	5′- AACGCAGGCTCCTCCACAGG
SEQ ID No. 725:	5′- CGGCTTAAGGTCAAACCAAC
SEQ ID No. 726:	5′- CCGGCTTAAGGTCAAACCAA
SEQ ID No. 727:	5′- CACCGGCTTAAGGTCAAACC
SEQ ID No. 728:	5′- ACCGGCTTAAGGTCAAACCA

SEQ ID No. 729: 5′- ACCCAACATCCAGCACACAT
SEQ ID No. 730: 5′- TCGCTGACCCGACCGTGGTC
SEQ ID No. 731: 5′- CGCTGACCCGACCGTGGTCG
SEQ ID No. 732: 5′- GACCCGACCGTGGTCGGCTG
SEQ ID No. 733: 5′- GCTGACCCGACCGTGGTCGG
SEQ ID No. 734: 5′- CTGACCCGACCGTGGTCGGC
SEQ ID No. 735: 5′- CAGGCGACTTGCGCCTTTGA
SEQ ID No. 736: 5′- TCATGCGGTATTAGCTCCAG
SEQ ID No. 737: 5′- ACTAGCTAATCGAACGCAGG
SEQ ID No. 738: 5′- CATGCGGTATTAGCTCCAGT
SEQ ID No. 739: 5′- CGCAGGCTCCTCCACAGGCG
SEQ ID No. 740: 5′- ACGCAGGCTCCTCCACAGGC
SEQ ID No. 741: 5′- CTCAGGTGTCATGCGGTATT
SEQ ID No. 742: 5′- CGCCTTTGACCCTCAGGTGT
SEQ ID No. 743: 5′- ACCCTCAGGTGTCATGCGGT
SEQ ID No. 744: 5′- CCTCAGGTGTCATGCGGTAT
SEQ ID No. 745: 5′- TTTGACCCTCAGGTGTCATG
SEQ ID No. 746: 5′- GACCCTCAGGTGTCATGCGG
SEQ ID No. 747: 5′- TGACCCTCAGGTGTCATGCG
SEQ ID No. 748: 5′- GCCTTTGACCCTCAGGTGTC
SEQ ID No. 749: 5′- TTGACCCTCAGGTGTCATGC
SEQ ID No. 750: 5′- CCCTCAGGTGTCATGCGGTA
SEQ ID No. 751: 5′- CCTTTGACCCTCAGGTGTCA
SEQ ID No. 752: 5′- CTTTGACCCTCAGGTGTCAT
SEQ ID No. 753: 5′- AGTTATCCCCCACCCATGGA
SEQ ID No. 754: 5′- CCAGCTATCGATCATCGCCT
SEQ ID No. 755: 5′- ACCAGCTATCGATCATCGCC
SEQ ID No. 756: 5′- CAGCTATCGATCATCGCCTT
SEQ ID No. 757: 5′- AGCTATCGATCATCGCCTTG
SEQ ID No. 758: 5′- GCTATCGATCATCGCCTTGG

SEQ ID No. 759: 5′- CTATCGATCATCGCCTTGGT
SEQ ID No. 760: 5′- TTCGTGCGACTTGCATGTGT
SEQ ID No. 761: 5′- TCGATCATCGCCTTGGTAGG
SEQ ID No. 762: 5′- ATCGATCATCGCCTTGGTAG
SEQ ID No. 763: 5′- CACAGGCGACTTGCGCCTTT
SEQ ID No. 764: 5′- CCACAGGCGACTTGCGCCTT
SEQ ID No. 765: 5′- TCCACAGGCGACTTGCGCCT
SEQ ID No. 766: 5′- TCCTCCACAGGCGACTTGCG
SEQ ID No. 767: 5′- CCTCCACAGGCGACTTGCGC
SEQ ID No. 768: 5′- CTCCACAGGCGACTTGCGCC
SEQ ID No. 769: 5′- ACAGGCGACTTGCGCCTTTG
SEQ ID No. 770: 5′- TCACCGGCTTAAGGTCAAAC
SEQ ID No. 771: 5′- CAACCCTCTCTCACACTCTA
SEQ ID No. 772: 5′- ACAACCCTCTCTCACACTCT
SEQ ID No. 773: 5′- CCACAACCCTCTCTCACACT
SEQ ID No. 774: 5′- AACCCTCTCTCACACTCTAG
SEQ ID No. 775: 5′- CACAACCCTCTCTCACACTC
SEQ ID No. 776: 5′- TCCACAACCCTCTCTCACAC
SEQ ID No. 777: 5′- TTCCACAACCCTCTCTCACA
SEQ ID No. 778: 5′- ACCCTCTCTCACACTCTAGT
SEQ ID No. 779: 5′- GAGCCAGGTTGCCGCCTTCG
SEQ ID No. 780: 5′- AGGTCAAACCAACTCCCATG
SEQ ID No. 781: 5′- ATGAGCCAGGTTGCCGCCTT
SEQ ID No. 782: 5′- TGAGCCAGGTTGCCGCCTTC
SEQ ID No. 783: 5′- AGGCTCCTCCACAGGCGACT
SEQ ID No. 784: 5′- CAGGCTCCTCCACAGGCGAC
SEQ ID No. 785: 5′- GCAGGCTCCTCCACAGGCGA
SEQ ID No. 786: 5′- TTCGCTCACCGGCTTAAGGT
SEQ ID No. 787: 5′- GTTCGCTCACCGGCTTAAGG
SEQ ID No. 788: 5′- GGTTCGCTCACCGGCTTAAG

SEQ ID No. 789:	5′- ATTCCACAACCCTCTCTCAC
SEQ ID No. 790:	5′- TGACCCGACCGTGGTCGGCT
SEQ ID No. 791:	5′- CCCTCTCTCACACTCTAGTC
SEQ ID No. 792:	5′- GAATTCCACAACCCTCTCTC
SEQ ID No. 793:	5′- AGCCAGGTTGCCGCCTTCGC
SEQ ID No. 794:	5′- GCCAGGTTGCCGCCTTCGCC
SEQ ID No. 795:	5′- GGAATTCCACAACCCTCTCT
SEQ ID No. 796:	5′- GGGAATTCCACAACCCTCTC
SEQ ID No. 797:	5′- AACGCAGGCTCCTCCACAGG
SEQ ID No. 798:	5′- CGGCTTAAGGTCAAACCAAC
SEQ ID No. 799:	5′- CCGGCTTAAGGTCAAACCAA
SEQ ID No. 800:	5′- CACCGGCTTAAGGTCAAACC
SEQ ID No. 801:	5′- ACCGGCTTAAGGTCAAACCA
SEQ ID No. 802:	5′- ACCCAACATCCAGCACACAT
SEQ ID No. 803:	5′- TCGCTGACCCGACCGTGGTC
SEQ ID No. 804:	5′- CGCTGACCCGACCGTGGTCG
SEQ ID No. 805:	5′- GACCCGACCGTGGTCGGCTG
SEQ ID No. 806:	5′- GCTGACCCGACCGTGGTCGG
SEQ ID No. 807:	5′- CTGACCCGACCGTGGTCGGC
SEQ ID No. 808:	5′- CAGGCGACTTGCGCCTTTGA
SEQ ID No. 809:	5′- TCATGCGGTATTAGCTCCAG
SEQ ID No. 810:	5′- ACTAGCTAATCGAACGCAGG
SEQ ID No. 811:	5′- CATGCGGTATTAGCTCCAGT
SEQ ID No. 812:	5′- CGCAGGCTCCTCCACAGGCG
SEQ ID No. 813:	5′- ACGCAGGCTCCTCCACAGGC
SEQ ID No. 814:	5′- CTCAGGTGTCATGCGGTATT
SEQ ID No. 815:	5′- CGCCTTTGACCCTCAGGTGT
SEQ ID No. 816:	5′- ACCCTCAGGTGTCATGCGGT
SEQ ID No. 817:	5′- CCTCAGGTGTCATGCGGTAT
SEQ ID No. 818:	5′- TTTGACCCTCAGGTGTCATG

SEQ ID No. 819: 5′- GACCCTCAGGTGTCATGCGG
SEQ ID No. 820: 5′- TGACCCTCAGGTGTCATGCG
SEQ ID No. 821: 5′- GCCTTTGACCCTCAGGTGTC
SEQ ID No. 822: 5′- TTGACCCTCAGGTGTCATGC
SEQ ID No. 823: 5′- CCCTCAGGTGTCATGCGGTA
SEQ ID No. 824: 5′- CCTTTGACCCTCAGGTGTCA
SEQ ID No. 825: 5′- CTTTGACCCTCAGGTGTCAT
SEQ ID No. 826: 5′- AGTTATCCCCCACCCATGGA
SEQ ID No. 827: 5′- CCAGCTATCGATCATCGCCT
SEQ ID No. 828: 5′- ACCAGCTATCGATCATCGCC
SEQ ID No. 829: 5′- CAGCTATCGATCATCGCCTT
SEQ ID No. 830: 5′- AGCTATCGATCATCGCCTTG
SEQ ID No. 831: 5′- GCTATCGATCATCGCCTTGG
SEQ ID No. 832: 5′- CTATCGATCATCGCCTTGGT
SEQ ID No. 833: 5′- TTCGTGCGACTTGCATGTGT
SEQ ID No. 834: 5′- TCGATCATCGCCTTGGTAGG
SEQ ID No. 835: 5′- ATCGATCATCGCCTTGGTAG
SEQ ID No. 836: 5′- CACAGGCGACTTGCGCCTTT
SEQ ID No. 837: 5′- CCACAGGCGACTTGCGCCTT
SEQ ID No. 838: 5′- TCCACAGGCGACTTGCGCCT
SEQ ID No. 839: 5′- TCCTCCACAGGCGACTTGCG
SEQ ID No. 840: 5′- CCTCCACAGGCGACTTGCGC
SEQ ID No. 841: 5′- CTCCACAGGCGACTTGCGCC
SEQ ID No. 842: 5′- ACAGGCGACTTGCGCCTTTG

The sequences SEQ ID No. 609 to SEQ ID No. 842 are particularly suitable for the simultanous detection of bacteria of the genera *Acetobacter, Gluconobacter* and *Gluconoacetobacter*.

e) Nucleic acid probe molecules, which specifically detect drink-spoiling bacilli:

SEQ ID No. 843: 5′- AGCCCCGGTTTCCCGGCGTT
SEQ ID No. 844: 5′- CGCCTTTCCTTTTTCCTCCA
SEQ ID No. 845: 5′- GCCCCGGTTTCCCGGCGTTA
SEQ ID No. 846: 5′- GCCGCCTTTCCTTTTTCCTC
SEQ ID No. 847: 5′- TAGCCCCGGTTTCCCGGCGT
SEQ ID No. 848: 5′- CCGGGTACCGTCAAGGCGCC
SEQ ID No. 849: 5′- AAGCCGCCTTTCCTTTTTCC
SEQ ID No. 850: 5′- CCCCGGTTTCCCGGCGTTAT
SEQ ID No. 851: 5′- CCGGCGTTATCCCAGTCTTA
SEQ ID No. 852: 5′- AGCCGCCTTTCCTTTTTCCT
SEQ ID No. 853: 5′- CCGCCTTTCCTTTTTCCTCC
SEQ ID No. 854: 5′- TTAGCCCCGGTTTCCCGGCG
SEQ ID No. 855: 5′- CCCGGCGTTATCCCAGTCTT
SEQ ID No. 856: 5′- GCCGGGTACCGTCAAGGCGC
SEQ ID No. 857: 5′- GGCCGGGTACCGTCAAGGCG
SEQ ID No. 858: 5′- TCCCGGCGTTATCCCAGTCT
SEQ ID No. 859: 5′- TGGCCGGGTACCGTCAAGGC
SEQ ID No. 860: 5′- GAAGCCGCCTTTCCTTTTTC
SEQ ID No. 861: 5′- CCCGGTTTCCCGGCGTTATC
SEQ ID No. 862: 5′- CGGCGTTATCCCAGTCTTAC
SEQ ID No. 863: 5′- GGCGTTATCCCAGTCTTACA
SEQ ID No. 864: 5′- GCGTTATCCCAGTCTTACAG
SEQ ID No. 865: 5′- CGGGTACCGTCAAGGCGCCG
SEQ ID No. 866: 5′- ATTAGCCCCGGTTTCCCGGC
SEQ ID No. 867: 5′- AAGGGGAAGGCCCTGTCTCC
SEQ ID No. 868: 5′- GGCCCTGTCTCCAGGGAGGT
SEQ ID No. 869: 5′- AGGCCCTGTCTCCAGGGAGG
SEQ ID No. 870: 5′- AAGGCCCTGTCTCCAGGGAG
SEQ ID No. 871: 5′- GCCCTGTCTCCAGGGAGGTC

SEQ ID No. 872: 5′- CGTTATCCCAGTCTTACAGG
SEQ ID No. 873: 5′- GGGTACCGTCAAGGCGCCGC
SEQ ID No. 874: 5′- CGGCAACAGAGTTTTACGAC
SEQ ID No. 875: 5′- GGGGAAGGCCCTGTCTCCAG
SEQ ID No. 876: 5′- AGGGGAAGGCCCTGTCTCCA
SEQ ID No. 877: 5′- GCAGCCGAAGCCGCCTTTCC
SEQ ID No. 878: 5′- TTCTTCCCCGGCAACAGAGT
SEQ ID No. 879: 5′- CGGCACTTGTTCTTCCCCGG
SEQ ID No. 880: 5′- GTTCTTCCCCGGCAACAGAG
SEQ ID No. 881: 5′- GGCACTTGTTCTTCCCCGGC
SEQ ID No. 882: 5′- GCACTTGTTCTTCCCCGGCA
SEQ ID No. 883: 5′- CACTTGTTCTTCCCCGGCAA
SEQ ID No. 884: 5′- TCTTCCCCGGCAACAGAGTT
SEQ ID No. 885: 5′- TTGTTCTTCCCCGGCAACAG
SEQ ID No. 886: 5′- ACTTGTTCTTCCCCGGCAAC
SEQ ID No. 887: 5′- TGTTCTTCCCCGGCAACAGA
SEQ ID No. 888: 5′- CTTGTTCTTCCCCGGCAACA
SEQ ID No. 889: 5′- ACGGCACTTGTTCTTCCCCG
SEQ ID No. 890: 5′- GTCCGCCGCTAACCTTTTAA
SEQ ID No. 891: 5′- CTGGCCGGGTACCGTCAAGG
SEQ ID No. 892: 5′- TCTGGCCGGGTACCGTCAAG
SEQ ID No. 893: 5′- TTCTGGCCGGGTACCGTCAA
SEQ ID No. 894: 5′- CAATGCTGGCAACTAAGGTC
SEQ ID No. 895: 5′- CGTCCGCCGCTAACCTTTTA
SEQ ID No. 896: 5′- CGAAGCCGCCTTTCCTTTTT
SEQ ID No. 897: 5′- CCGAAGCCGCCTTTCCTTTT
SEQ ID No. 898: 5′- GCCGAAGCCGCCTTTCCTTT
SEQ ID No. 899: 5′- AGCCGAAGCCGCCTTTCCTT
SEQ ID No. 900: 5′- ACCGTCAAGGCGCCGCCCTG
SEQ ID No. 901: 5′- CCGTGGCTTTCTGGCCGGGT

SEQ ID No. 902: 5′- GCTTTCTGGCCGGGTACCGT
SEQ ID No. 903: 5′- GCCGTGGCTTTCTGGCCGGG
SEQ ID No. 904: 5′- GGCTTTCTGGCCGGGTACCG
SEQ ID No. 905: 5′- CTTTCTGGCCGGGTACCGTC
SEQ ID No. 906: 5′- TGGCTTTCTGGCCGGGTACC
SEQ ID No. 907: 5′- GTGGCTTTCTGGCCGGGTAC
SEQ ID No. 908: 5′- CGTGGCTTTCTGGCCGGGTA
SEQ ID No. 909: 5′- TTTCTGGCCGGGTACCGTCA
SEQ ID No. 910: 5′- GGGAAGGCCCTGTCTCCAGG
SEQ ID No. 911: 5′- CGAAGGGGAAGGCCCTGTCT
SEQ ID No. 912: 5′- CCGAAGGGGAAGGCCCTGTC
SEQ ID No. 913: 5′- GAAGGGGAAGGCCCTGTCTC
SEQ ID No. 914: 5′- GGCGCCGCCCTGTTCGAACG
SEQ ID No. 915: 5′- AGGCGCCGCCCTGTTCGAAC
SEQ ID No. 916: 5′- AAGGCGCCGCCCTGTTCGAA
SEQ ID No. 917: 5′- CCCGGCAACAGAGTTTTACG
SEQ ID No. 918: 5′- CCCCGGCAACAGAGTTTTAC
SEQ ID No. 919: 5′- CCATCTGTAAGTGGCAGCCG
SEQ ID No. 920: 5′- TCTGTAAGTGGCAGCCGAAG
SEQ ID No. 921: 5′- CTGTAAGTGGCAGCCGAAGC
SEQ ID No. 922: 5′- CCCATCTGTAAGTGGCAGCC
SEQ ID No. 923: 5′- TGTAAGTGGCAGCCGAAGCC
SEQ ID No. 924: 5′- CATCTGTAAGTGGCAGCCGA
SEQ ID No. 925: 5′- ATCTGTAAGTGGCAGCCGAA
SEQ ID No. 926: 5′- CAGCCGAAGCCGCCTTTCCT
SEQ ID No. 927: 5′- GGCAACAGAGTTTTACGACC
SEQ ID No. 928: 5′- CCGGCAACAGAGTTTTACGA
SEQ ID No. 929: 5′- TTCCCCGGCAACAGAGTTTT
SEQ ID No. 930: 5′- CTTCCCCGGCAACAGAGTTT
SEQ ID No. 931: 5′- TCCCCGGCAACAGAGTTTTA

SEQ ID No. 932: 5′- CCGTCCGCCGCTAACCTTTT

The sequences SEQ ID No. 843 to SEQ ID No. 932 are particularly suitable for the detection of *Bacillus coagulans.*

f) Nucleic acid probe molecules which specifically detect drink-spoiling Alicyclobacilli:

SEQ ID No. 933: 5′- CTTCCTCCGACTTACGCCGG
SEQ ID No. 934: 5′- CCTCCGACTTACGCCGGCAG
SEQ ID No. 935: 5′- TTCCTCCGACTTACGCCGGC
SEQ ID No. 936: 5′- TCCTCCGACTTACGCCGGCA
SEQ ID No. 937: 5′- TCCGACTTACGCCGGCAGTC
SEQ ID No. 938: 5′- CCGACTTACGCCGGCAGTCA
SEQ ID No. 939: 5′- GCCTTCCTCCGACTTACGCC
SEQ ID No. 940: 5′- CCTTCCTCCGACTTACGCCG
SEQ ID No. 941: 5′- GCTCTCCCCGAGCAACAGAG
SEQ ID No. 942: 5′- CTCTCCCCGAGCAACAGAGC
SEQ ID No. 943: 5′- CGCTCTCCCCGAGCAACAGA
SEQ ID No. 944: 5′- CTCCGACTTACGCCGGCAGT
SEQ ID No. 945: 5′- TCTCCCCGAGCAACAGAGCT
SEQ ID No. 946: 5′- CGACTTACGCCGGCAGTCAC
SEQ ID No. 947: 5′- TCGGCACTGGGGTGTGTCCC
SEQ ID No. 948: 5′- GGCACTGGGGTGTGTCCCCC
SEQ ID No. 949: 5′- CTGGGGTGTGTCCCCCCAAC
SEQ ID No. 950: 5′- CACTGGGGTGTGTCCCCCCA
SEQ ID No. 951: 5′- ACTGGGGTGTGTCCCCCCAA
SEQ ID No. 952: 5′- GCACTGGGGTGTGTCCCCCC
SEQ ID No. 953: 5′- TGGGGTGTGTCCCCCCAACA
SEQ ID No. 954: 5′- CACTCCAGACTTGCTCGACC

SEQ ID No. 955: 5′- TCACTCCAGACTTGCTCGAC
SEQ ID No. 956: 5′- CGGCACTGGGGTGTGTCCCC
SEQ ID No. 957: 5′- CGCCTTCCTCCGACTTACGC
SEQ ID No. 958: 5′- CTCCCCGAGCAACAGAGCTT
SEQ ID No. 959: 5′- ACTCCAGACTTGCTCGACCG
SEQ ID No. 960: 5′- CCCATGCCGCTCTCCCCGAG
SEQ ID No. 961: 5′- CCATGCCGCTCTCCCCGAGC
SEQ ID No. 962: 5′- CCCCATGCCGCTCTCCCCGA
SEQ ID No. 963: 5′- TCACTCGGTACCGTCTCGCA
SEQ ID No. 964: 5′- CATGCCGCTCTCCCCGAGCA
SEQ ID No. 965: 5′- ATGCCGCTCTCCCCGAGCAA
SEQ ID No. 966: 5′- TTCGGCACTGGGGTGTGTCC
SEQ ID No. 967: 5′- TGCCGCTCTCCCCGAGCAAC
SEQ ID No. 968: 5′- TTCACTCCAGACTTGCTCGA
SEQ ID No. 969: 5′- CCCGCAAGAAGATGCCTCCT
SEQ ID No. 970: 5′- AGAAGATGCCTCCTCGCGGG
SEQ ID No. 971: 5′- AAGAAGATGCCTCCTCGCGG
SEQ ID No. 972: 5′- CGCAAGAAGATGCCTCCTCG
SEQ ID No. 973: 5′- AAGATGCCTCCTCGCGGGCG
SEQ ID No. 974: 5′- CCGCAAGAAGATGCCTCCTC
SEQ ID No. 975: 5′- GAAGATGCCTCCTCGCGGGC
SEQ ID No. 976: 5′- CCCCGCAAGAAGATGCCTCC
SEQ ID No. 977: 5′- CAAGAAGATGCCTCCTCGCG
SEQ ID No. 978: 5′- TCCTTCGGCACTGGGGTGTG
SEQ ID No. 979: 5′- CCGCTCTCCCCGAGCAACAG
SEQ ID No. 980: 5′- TGCCTCCTCGCGGGCGTATC
SEQ ID No. 981: 5′- GACTTACGCCGGCAGTCACC
SEQ ID No. 982: 5′- GGCTCCTCTCTCAGCGGCCC
SEQ ID No. 983: 5′- CCTTCGGCACTGGGGTGTGT
SEQ ID No. 984: 5′- GGGGTGTGTCCCCCCAACAC

SEQ ID No. 985: 5′- GCCGCTCTCCCCGAGCAACA
SEQ ID No. 986: 5′- AGATGCCTCCTCGCGGGCGT
SEQ ID No. 987: 5′- CACTCGGTACCGTCTCGCAT
SEQ ID No. 988: 5′- CTCACTCGGTACCGTCTCGC
SEQ ID No. 989: 5′- GCAAGAAGATGCCTCCTCGC
SEQ ID No. 990: 5′- CTCCAGACTTGCTCGACCGC
SEQ ID No. 991: 5′- TTACGCCGGCAGTCACCTGT
SEQ ID No. 992: 5′- CTTCGGCACTGGGGTGTGTC
SEQ ID No. 993: 5′- CTCGCGGGCGTATCCGGCAT
SEQ ID No. 994: 5′- GCCTCCTCGCGGGCGTATCC
SEQ ID No. 995: 5′- ACTCGGTACCGTCTCGCATG
SEQ ID No. 996: 5′- GATGCCTCCTCGCGGGCGTA
SEQ ID No. 997: 5′- GGGTGTGTCCCCCCAACACC
SEQ ID No. 998: 5′- ACTTACGCCGGCAGTCACCT
SEQ ID No. 999: 5′- CTTACGCCGGCAGTCACCTG
SEQ ID No. 1000: 5′- ATGCCTCCTCGCGGGCGTAT
SEQ ID No. 1001: 5′- GCGCCGCGGGCTCCTCTCTC
SEQ ID No. 1002: 5′- GGTGTGTCCCCCCAACACCT
SEQ ID No. 1003: 5′- GTGTGTCCCCCCAACACCTA
SEQ ID No. 1004: 5′- CCTCGCGGGCGTATCCGGCA
SEQ ID No. 1005: 5′- CCTCACTCGGTACCGTCTCG
SEQ ID No. 1006: 5′- TCCTCACTCGGTACCGTCTC
SEQ ID No. 1007: 5′- TCGCGGGCGTATCCGGCATT
SEQ ID No. 1008: 5′- TTTCACTCCAGACTTGCTCG
SEQ ID No. 1009: 5′- TACGCCGGCAGTCACCTGTG
SEQ ID No. 1010: 5′- TCCAGACTTGCTCGACCGCC
SEQ ID No. 1011: 5′- CTCGGTACCGTCTCGCATGG
SEQ ID No. 1012: 5′- CGCGGGCGTATCCGGCATTA
SEQ ID No. 1013: 5′- GCGTATCCGGCATTAGCGCC
SEQ ID No. 1014: 5′- GGGCTCCTCTCTCAGCGGCC

SEQ ID No. 1015: 5′- TCCCCGAGCAACAGAGCTTT
SEQ ID No. 1016: 5′- CCCCGAGCAACAGAGCTTTA
SEQ ID No. 1017: 5′- CCGAGCAACAGAGCTTTACA
SEQ ID No. 1018: 5′- CCATCCCATGGTTGAGCCAT
SEQ ID No. 1019: 5′- GTGTCCCCCCAACACCTAGC
SEQ ID No. 1020: 5′- GCGGGCGTATCCGGCATTAG
SEQ ID No. 1021: 5′- CGAGCGGCTTTTTGGGTTTC
SEQ ID No. 1022: 5′- CTTTCACTCCAGACTTGCTC
SEQ ID No. 1023: 5′- TTCCTTCGGCACTGGGGTGT
SEQ ID No. 1024: 5′- CCGCCTTCCTCCGACTTACG
SEQ ID No. 1025: 5′- CCCGCCTTCCTCCGACTTAC
SEQ ID No. 1026: 5′- CCTCCTCGCGGGCGTATCCG
SEQ ID No. 1027: 5′- TCCTCGCGGGCGTATCCGGC
SEQ ID No. 1028: 5′- CATTAGCGCCCGTTTCCGGG
SEQ ID No. 1029: 5′- GCATTAGCGCCCGTTTCCGG
SEQ ID No. 1030: 5′- GGCATTAGCGCCCGTTTCCG
SEQ ID No. 1031: 5′- GTCTCGCATGGGGCTTTCCA
SEQ ID No. 1032: 5′- GCCATGGACTTTCACTCCAG
SEQ ID No. 1033: 5′- CATGGACTTTCACTCCAGAC

The sequences SEQ ID No. 933 to SEQ ID No. 1033 are particularly suitable for the detection of bacteria of the genus *Alicyclobacillus*.

SEQ ID No. 1034: 5′- CCTTCCTCCGGCTTACGCCGGC
SEQ ID No. 1035: 5′- CCTTCCTCCGACTTGCGCCGGC
SEQ ID No. 1036: 5′- CCTTCCTCCGACTTTCACCGGC

The nucleic acid probe molecules according to SEQ ID No. 1034 to SEQ ID No. 1036 are used as unlabelled competitor probes for the detection of bacteria of the genus *Alicyclobacillus* in combination with the oligonucleotide probe according to

SEQ ID No. 933, in order to prevent the binding of the labelled oligonucleotide probe specific for bacteria of the genus *Alicyclobacillus* to nucleic acid sequences which are not specific for bacteria of the genus *Alicyclobacillus*.

SEQ ID No. 1037: 5′- ACCGTCTCACAAGGAGCTTT
SEQ ID No. 1038: 5′- TACCGTCTCACAAGGAGCTT
SEQ ID No. 1039: 5′- GTACCGTCTCACAAGGAGCT
SEQ ID No. 1040: 5′- GCCTACCCGTGTATTATCCG
SEQ ID No. 1041: 5′- CCGTCTCACAAGGAGCTTTC
SEQ ID No. 1042: 5′- CTACCCGTGTATTATCCGGC
SEQ ID No. 1043: 5′- GGTACCGTCTCACAAGGAGC
SEQ ID No. 1044: 5′- CGTCTCACAAGGAGCTTTCC
SEQ ID No. 1045: 5′- TCTCACAAGGAGCTTTCCAC
SEQ ID No. 1046: 5′- TACCCGTGTATTATCCGGCA
SEQ ID No. 1047: 5′- GTCTCACAAGGAGCTTTCCA
SEQ ID No. 1048: 5′- ACCCGTGTATTATCCGGCAT
SEQ ID No. 1049: 5′- CTCGGTACCGTCTCACAAGG
SEQ ID No. 1050: 5′- CGGTACCGTCTCACAAGGAG
SEQ ID No. 1051: 5′- ACTCGGTACCGTCTCACAAG
SEQ ID No. 1052: 5′- CGGCTGGCTCCATAACGGTT
SEQ ID No. 1053: 5′- ACAAGTAGATGCCTACCCGT
SEQ ID No. 1054: 5′- TGGCTCCATAACGGTTACCT
SEQ ID No. 1055: 5′- CAAGTAGATGCCTACCCGTG
SEQ ID No. 1056: 5′- CACAAGTAGATGCCTACCCG
SEQ ID No. 1057: 5′- GGCTCCATAACGGTTACCTC
SEQ ID No. 1058: 5′- ACACAAGTAGATGCCTACCC
SEQ ID No. 1059: 5′- CTGGCTCCATAACGGTTACC
SEQ ID No. 1060: 5′- GCTGGCTCCATAACGGTTAC
SEQ ID No. 1061: 5′- GGCTGGCTCCATAACGGTTA
SEQ ID No. 1062: 5′- GCTCCATAACGGTTACCTCA

SEQ ID No. 1063: 5′- AAGTAGATGCCTACCCGTGT
SEQ ID No. 1064: 5′- CTCCATAACGGTTACCTCAC
SEQ ID No. 1065: 5′- TGCCTACCCGTGTATTATCC
SEQ ID No. 1066: 5′- TCGGTACCGTCTCACAAGGA
SEQ ID No. 1067: 5′- CTCACAAGGAGCTTTCCACT
SEQ ID No. 1068: 5′- GTAGATGCCTACCCGTGTAT
SEQ ID No. 1069: 5′- CCTACCCGTGTATTATCCGG
SEQ ID No. 1070: 5′- CACTCGGTACCGTCTCACAA
SEQ ID No. 1071: 5′- CTCAGCGATGCAGTTGCATC
SEQ ID No. 1072: 5′- AGTAGATGCCTACCCGTGTA
SEQ ID No. 1073: 5′- GCGGCTGGCTCCATAACGGT
SEQ ID No. 1074: 5′- CCAAAGCAATCCCAAGGTTG
SEQ ID No. 1075: 5′- TCCATAACGGTTACCTCACC
SEQ ID No. 1076: 5′- CCCGTGTATTATCCGGCATT
SEQ ID No. 1077: 5′- TCTCAGCGATGCAGTTGCAT
SEQ ID No. 1078: 5′- CCATAACGGTTACCTCACCG
SEQ ID No. 1079: 5′- TCAGCGATGCAGTTGCATCT
SEQ ID No. 1080: 5′- GGCGGCTGGCTCCATAACGG
SEQ ID No. 1081: 5′- AAGCAATCCCAAGGTTGAGC
SEQ ID No. 1082: 5′- TCACTCGGTACCGTCTCACA
SEQ ID No. 1083: 5′- CCGAGTGTTATTCCAGTCTG
SEQ ID No. 1084: 5′- CACAAGGAGCTTTCCACTCT
SEQ ID No. 1085: 5′- ACAAGGAGCTTTCCACTCTC
SEQ ID No. 1086: 5′- TCACAAGGAGCTTTCCACTC
SEQ ID No. 1087: 5′- CAGCGATGCAGTTGCATCTT
SEQ ID No. 1088: 5′- CAAGGAGCTTTCCACTCTCC
SEQ ID No. 1089: 5′- CCAGTCTGAAAGGCAGATTG
SEQ ID No. 1090: 5′- CAGTCTGAAAGGCAGATTGC
SEQ ID No. 1091: 5′- CGGCGGCTGGCTCCATAACG
SEQ ID No. 1092: 5′- CCTCTCTCAGCGATGCAGTT

SEQ ID No. 1093: 5′- CTCTCTCAGCGATGCAGTTG
SEQ ID No. 1094: 5′- TCTCTCAGCGATGCAGTTGC
SEQ ID No. 1095: 5′- CTCTCAGCGATGCAGTTGCA
SEQ ID No. 1096: 5′- CAATCCCAAGGTTGAGCCTT
SEQ ID No. 1097: 5′- AATCCCAAGGTTGAGCCTTG
SEQ ID No. 1098: 5′- AGCAATCCCAAGGTTGAGCC
SEQ ID No. 1099: 5′- CTCACTCGGTACCGTCTCAC
SEQ ID No. 1100: 5′- GCAATCCCAAGGTTGAGCCT
SEQ ID No. 1101: 5′- GCCTTGGACTTTCACTTCAG
SEQ ID No. 1102: 5′- CATAACGGTTACCTCACCGA
SEQ ID No. 1103: 5′- CTCCTCTCTCAGCGATGCAG
SEQ ID No. 1104: 5′- TCGGCGGCTGGCTCCATAAC
SEQ ID No. 1105: 5′- AGTCTGAAAGGCAGATTGCC
SEQ ID No. 1106: 5′- TCCTCTCTCAGCGATGCAGT
SEQ ID No. 1107: 5′- CCCAAGGTTGAGCCTTGGAC
SEQ ID No. 1108: 5′- ATAACGGTTACCTCACCGAC
SEQ ID No. 1109: 5′- TCCCAAGGTTGAGCCTTGGA
SEQ ID No. 1110: 5′- ATTATCCGGCATTAGCACCC
SEQ ID No. 1111: 5′- CTACGTGCTGGTAACACAGA
SEQ ID No. 1112: 5′- GCCGCTAGCCCCGAAGGGCT
SEQ ID No. 1113: 5′- CTAGCCCCGAAGGGCTCGCT
SEQ ID No. 1114: 5′- CGCTAGCCCCGAAGGGCTCG
SEQ ID No. 1115: 5′- AGCCCCGAAGGGCTCGCTCG
SEQ ID No. 1116: 5′- CCGCTAGCCCCGAAGGGCTC
SEQ ID No. 1117: 5′- TAGCCCCGAAGGGCTCGCTC
SEQ ID No. 1118: 5′- GCTAGCCCCGAAGGGCTCGC
SEQ ID No. 1119: 5′- GCCCCGAAGGGCTCGCTCGA
SEQ ID No. 1120: 5′- ATCCCAAGGTTGAGCCTTGG
SEQ ID No. 1121: 5′- GAGCCTTGGACTTTCACTTC
SEQ ID No. 1122: 5′- CAAGGTTGAGCCTTGGACTT

SEQ ID No. 1123: 5′- GAGCTTTCCACTCTCCTTGT
SEQ ID No. 1124: 5′- CCAAGGTTGAGCCTTGGACT
SEQ ID No. 1125: 5′- CGGGCTCCTCTCTCAGCGAT
SEQ ID No. 1126: 5′- GGAGCTTTCCACTCTCCTTG
SEQ ID No. 1127: 5′- GGGCTCCTCTCTCAGCGATG
SEQ ID No. 1128: 5′- TCTCCTTGTCGCTCTCCCCG
SEQ ID No. 1129: 5′- TCCTTGTCGCTCTCCCCGAG
SEQ ID No. 1130: 5′- AGCTTTCCACTCTCCTTGTC
SEQ ID No. 1131: 5′- CCACTCTCCTTGTCGCTCTC
SEQ ID No. 1132: 5′- GGCTCCTCTCTCAGCGATGC
SEQ ID No. 1133: 5′- CCTTGTCGCTCTCCCCGAGC
SEQ ID No. 1134: 5′- CACTCTCCTTGTCGCTCTCC
SEQ ID No. 1135: 5′- ACTCTCCTTGTCGCTCTCCC
SEQ ID No. 1136: 5′- CTCTCCTTGTCGCTCTCCCC
SEQ ID No. 1137: 5′- GCGGGCTCCTCTCTCAGCGA
SEQ ID No. 1138: 5′- GGCTCCATCATGGTTACCTC

The sequences SEQ ID No. 1037 to SEQ ID No. 1138 are particularly suitable for the detection of *Alicyclobacillus acidoterrestris*.

SEQ ID No. 1139: 5′- CCGTCTCCTAAGGAGCTTTCCA

The nucleic acid probe molecule according to SEQ ID No. 1139 is used as unlabelled competitor probe for the detection of *Alicyclobacillus acidoterrestris* in combination with the oligonucleotide probe according to SEQ ID No. 1044, in order to prevent the binding of the labelled oligonucleotide probe specific for *Alicyclobacillus acidoterrestris* to nucleic acid sequences which are not specific for *Alicyclobacillus acidoterrestris*.

SEQ ID No. 1140: 5′- TCCCTCCTTAACGGTTACCTCA

SEQ ID No. 1141: 5′- TGGCTCCATAA(A/T)GGTTACCTCA

The nucleic acid probe molecules according to SEQ ID No. 1140 to SEQ ID No. 1141 are used as unlabelled competitor probe for the detection of *Alicyclobacillus acidoterrestris* in combination with the oligonucleotide probe according to SEQ ID No. 1057, in order to prevent the binding of the labelled oligonucleotide probe specific for *Alicyclobacillus acidoterrestris,* to nucleic acid sequences which are not specific for *Alicyclobacillus acidoterrestris.*

SEQ ID No. 1142: 5′- CTTCCTCCGGCTTGCGCCGG
SEQ ID No. 1143: 5′- CGCTCTTCCCGA(G/T)TGACTGA
SEQ ID No. 1144: 5′- CCTCGGGCTCCTCCATC(A/T)GC

The sequences SEQ ID No. 1142 to SEQ ID No. 1144 are particularly suitable for the simultanous detection of *Alicyclobacillus cycloheptanicus* and *A. herbarius.*

A further subject of the invention are derivatives of the above oligonucleotide sequences, demonstrating specific hybridization with target nucleic acid sequences of the respective microorganism despite deviations in sequence and/or length, and which are therefore suitable for use in a method according to the invention and ensure the the specific detection of the respective micororganism. These derivatives especially include:

a) nucleic acid molecules which (i) are identical with respect to the bases to one of the above oligonucleotide sequences (SEQ ID No. 1, 5 to 146, 148 to 154, 157 to 160, 163 to 1033, 1037 to 1138, 1142 to 1144) to at least 80%, preferably to at least 90% particularly preferred to at least 92%, 94%, 96%, or (ii) differ from the above oligonucleotide sequences by one or more deletions and/or additions and which allow for a specific hybridization with nucleic acid sequences of drink-spoiling yeasts of the genera *Zygosaccharomyces, Hanseniaspora, Candida,*

Brettanomyces, Dekkera, Pichia, Saccharomyces and *Saccharomycodes* and in particular of the species *Zygosaccharomyces bailii*, *Z. mellis*, *Z. rouxii*, *Z. bisporus*, *Z. fermentati*, *Z. microellipsoides*, *Hanseniaspora uvarum*, *Candida intermedia*, *C. crusei (Issatchenkia orientalis)*, *C. parapsilosis*, *Brettanomyces bruxellensis*, *B. naardenensis*, *Dekkera anomala*, *Pichia membranaefaciens*, *P. minuta*, *P. anomala*, *Saccharomyces exiguus*, *S. cerevisiae*, *Saccharomycodes ludwigii* or of the drink-spoiling molds of the genera Mucor, Byssochlamys, Neosartorya, Aspergillus und Talaromyces, in particular of the species *Mucor racemosus*, *Byssochlamys nivea*, *Neosartorya fischeri*, *Aspergillus fumigatus und A. fischeri*, *Talaromyces flavus*, *T. bacillisporus* and *T. flavus* or of the drink-spoiling bacteria of the genera *Lactobacillus, Leuconostoc, Oenococcus, Weissella, Lactococcus, Acetobacter, Gluconobacter, Gluconoacetobacter, Bacillus* and *Alicyclobacillus*, in particular of the species *Lactobacillus collinoides*, *Leuconostoc mesenteroides*, *L. pseudomesenteroides*, *Oenococcus oeni*, *Bacillus coagulans*, *Alicyclobacillus ssp.*, *A. acidoterrestris*, *A. cycloheptanicus* and *A. herbarius.* In this context "specific hybridization" means that under the hybridization conditions described here or those known to the person skilled in the art in relation to *in situ* hybridization techniques, only the ribosomal RNA of the target organisms binds to the oligonucleotide, but not the rRNA of non-target microrganisms.

b) nucleic acid molecules which specifically hybridize under stringent conditions to a sequence complementary to the nucleic acid molecules mentioned in a) or to one of the probes SEQ ID No. 1, 5 to 146, 148 to 154, 157 to 160, 163 to 1033, 1037 to 1138, 1142 to 1144.

c) Nucleic acid molecules comprising an oligonucleotide sequence of SEQ ID No. 1, 5 to 146, 148 to 154, 157 to 160, 163 to 1033, 1037 to 1138, 1142 to 1144 or the sequence of a nucleic acid molecule according to a) or b) and having at least one further nucleotide in addition to the mentioned sequences and their

derivatives, respectively, according to a) or b) and allowing specific hybridization with nucleic acid sequences of target organisms.

A further subject of the invention are also derivatives of the above competitor probe sequences, showing specific hybridizations with target nucleic acid sequences of the respective non-target genrera and species, respectively, despite variations in sequence and/or length, and which therefore prevent the binding of the oligonucleotide probe to the nucleic acid sequences of the genera and species, respectively, not to be detected. They are suitable for use in a method according to the invention and ensure a specific detection of the respective microorganism. These derivatives especially include

a) nucleic acid molecules which (i) are identical in terms of bases to one of the above oligonucleotide sequences (SEQ ID No. 2 to 4, 147, 155 to 156, 161 to 162, 1034 to 1036, 1139 to 1141) to at least 80%, preferably to at least 90%, particularly preferably to at least 92%, 94%, 96%, or (ii) differ from the above oligonucleotide sequences by one or more deletions and/or additions and which inhibit a specific hybridization of a specific oligonucleotide probe to nucleic acid sequences of a microorganism not to be detected.

b) Nucleic acid molecules which specifically hybridize to a sequence complementary to the nucleic acid molecules mentioned in a) or to one of the probes SEQ ID No. 2 to 4, 147, 155 to 156, 161 to 162, 1034 to 1036, 1139 to 1141 under stringent conditions.

c) Nucleic acid molecules comprising an oligonucleotide sequence of SEQ ID No. 2 to 4, 147, 155 to 156, 161 to 162, 1034 to 1036, 1139 to 1141 or the sequence of a nucleic acid molecule according to a) or b) and having at least one further nucleotide in addition to the mentioned sequences and their derivatives, respectively, according

to a) or b) and prevent the binding of a specific oligonucleotide probe to the nucleic acid sequence of a non-target microorganism.

The degree of sequence identity of a nucleic acid probe molecule to the oligonucleotide probes having SEQ ID No. 1 to SEQ ID No. 1144 can be determined using the usual algorithms. In this respect, for example, the program for determining the sequence identity available under http://www.ncbi.nlm.nih.gov/BLAST (on this page for example the link "Standard nucleotide-nucleotide BLAST [blastn]") is suitable.

In the present invention „hybridization" can have the same meaning as „complementary". The present invention also comprises those oligonucleotides, which hybridize to the (theoretical) antisense strand of one of the inventive oligonucleotides including the derivatives of the present invention of SEQ ID No. 1 bis SEQ ID No. 1144.

The term "stringent conditions" generally means conditions under which a nucleic acid sequence preferentially hybridizes to its target sequence and to a clearly lower extent, or not at all, to other sequences. Stringent conditions are partly sequence-dependent and will vary under different circumstances. Longer sequences hybridize specifically at higher temperatures. In general, stringent conditions are selected in such a way that the temperature is approximately 5°C below the thermal melting point (T_m) for the specific sequence at a defined ionic strength, pH and nucleic acid concentration. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probe molecules complementary to the target sequence hybridize to the target sequence in the steady state.

The nucleic acid probe molecules of the present invention may be used within the detection method with various hybridization solutions. Various organic solvents may be used in concentrations of 0-80%. By keeping stringent hybridization conditions, it

is guaranteed that the nucleic acid probe molecule indeed hybridizes to the target sequence. Moderate conditions within the meaning of the invention are e.g. 0% formamide in a hybridization buffer as described below. Stringent conditions within the meaning of the invention are for example 20 % to 80 % formamide in the hybridization buffer.

Within the method according to the invention for the specific detection of yeasts of the genera *Zygosaccharomyces, Hanseniaspora, Candida, Brettanomyces, Dekkera, Pichia, Saccharomyces* and *Saccharomycodes*, in particular of the species *Zygosaccharomyces bailii, Z. mellis, Z. rouxii, Z. bisporus, Z. fermentati, Z. microellipsoides, Hanseniaspora uvarum, Candida intermedia, C. crusei (Issatchenkia orientalis), C. parapsilosis, Brettanomyces bruxellensis, B. naardenensis, Dekkera anomala, Pichia membranaefaciens, P. minuta, P. anomala, Saccharomyces exiguus, S. cerevisiae, Saccharomycodes ludwigii* a typical hybridization solution contains 0%-80% formamide, preferably 20%-60% formamide, particularly preferably 40% formamide. In addition, it has a salt concentration of 0.1 mol/l – 1.5 mol/l, preferably of 0.7 mol/l – 1.0 mol/l, and particularly preferably of 0.9 mol/l, whereby the salt preferably being sodium chloride. Further, the hybridization solution usually comprises a detergent, such as for instance sodium dodecyl sulfate (SDS) in a concentration of 0.001% - 0.2%, preferably in a concentration of 0.005% – 0.05%, particularly preferably in a concentration of 0.01%. For buffering the hybridization solution, various compounds such as Tris-HCl, sodium citrate, PIPES or HEPES may be used, which are usually used in concentrations of 0.01 – 0.1 mol/l, preferably of 0.01 to 0.05 mol/l, in a pH range of 6.0 – 9.0, preferably 7.0 to 8.0. The particularly preferred embodiment of the hybridization solution in accordance with the invention contains 0.02 mol/l Tris-HCl, pH 8.0.

Within the method according to the invention for the specific detection of molds of the genera *Mucor, Byssochlamys, Neosartorya, Aspergillus* and *Talaromyces*, in

particular of the species *Mucor racemosus*, *Byssochlamys nivea*, *Neosartorya fischeri*, *Aspergillus fumigatus und A. fischeri*, *Talaromyces flavus*, *T. bacillisporus* and *T. flavus,* a typical hybridization solution contains 0%-80% formamide, preferably 10%-60% formamide, particularly preferably 20% formamide. In addition, it has a salt concentration of 0.1 mol/l – 1.5 mol/l, preferably of 0.7 mol/l – 1.0 mol/l, and particularly preferably of 0.9 mol/l, whereby the salt preferably being sodium chloride. Further, the hybridization solution usually comprises a detergent, such as for instance sodium dodecyl sulfate (SDS) at a concentration of 0.001% - 0.2%, preferably at a concentration of 0.005 – 0.05%, particularly preferably at a concentration of 0.01%. For buffering the hybridization solution, various compounds such as Tris-HCl, sodium citrate, PIPES or HEPES may be used, which are usually used in concentrations of 0.01 – 0.1 mol/l, preferably of 0.01 to 0.05 mol/l, in a pH range of 6.0 – 9.0, preferably 7.0 to 8.0. The particularly preferred embodiment of the hybridization solution in accordance with the invention contains 0.02 mol/l Tris-HCl, pH 8.0.

Within the method according to the invention for the specific detection of bacteria of the genera *Lactobacillus, Leuconostoc, Oenococcus, Weissella, Lactococcus, Acetobacter, Gluconobacter, Gluconoacetobacter, Bacillus* and *Alicyclobacillus,* in particular of the species *Lactobacillus collinoides*, *Leuconostoc mesenteroides*, *L. pseudomesenteroides*, *Oenococcus oeni*, *Bacillus coagulans*, *Alicyclobacillus ssp.*, *A. acidoterrestris*, *A. cycloheptanicus* and *A. herbarius,* a typical hybridization solution contains 0%-80% formamide, preferably 10%-60% formamide, particularly preferably 20% formamide. In addition, it has a salt concentration of 0.1 mol/l – 1.5 mol/l, preferably of 0.7 mol/l – 1.0 mol/l, and particularly preferably of 0.9 mol/l, whereby the salt preferably being sodium chloride. Further, the hybridization solution usually comprises a detergent, such as for instance sodium dodecyl sulfate (SDS) at a concentration of 0.001% - 0.2%, preferably at a concentration of 0.005% – 0.05%, particularly preferably at a concentration of 0.01%. For buffering the hybridization solution, various compounds such as Tris-HCl, sodium citrate, PIPES

or HEPES may be used, which are usually used in concentrations of 0.01 – 0.1 mol/l, preferably of 0.01 to 0.05 mol/l, in a pH range of 6.0 – 9.0, preferably 7.0 to 8.0. The particularly preferred embodiment of the hybridization solution in accordance with the invention contains 0.02 mol/l Tris-HCl, pH 8.0.

It shall be understood that the one skilled in the art can select the specified concentrations of the constituents of the hybridization buffer in such a way that the desired stringency of the hybridization reaction is achieved. Particularly preferred embodiments are related from stringent to particularly stringent hybridization conditions. Using these stringent conditions the one skilled in the art can determine whether a particular nucleic acid molecule allows the specific detection of nucleic acid sequences of target organisms and may thus be reliably used within the invention.

The concentration of the nucleic acid probe in the hybridization buffer depends on the kind of label and on the number of target structures. In order to allow rapid and efficient hybridization, the number of nucleic acid probe molecules should exceed the number of target structures by several orders of magnitude. However, it has to be taken into consideration that in fluorescence *in situ*-hybridization (FISH) too high levels of fluorescencently labelled nucleic acid probe molecules result in increased background fluorescence. The concentration of the nucleic acid probe molecules should therefore be in the range between 0.5 and 500 ng/μl. Within the method of the present invention the preferred nucleic acid probe concentration is between 1.0 and 10 ng for each nucleic acid probe molecule used per μl of hybridization solution. The volume of hybridization solution used should be between 8 μl and 100 ml, in a particularly preferred embodiment of the method of present invention it is 30 μl.

The concentration of the competitor probe in the hybridization buffer depends on the number of target structures. In order to allow rapid and efficient hybridization, the number of competitor probes should exceed the number of target structures by

several orders of magnitude. The concentration of the competitor probe molecules should therefore be in a range between 0.5 and 500 ng/μl. Within the method of the present invention the preferred concentration is between 1.0 and 10 ng for each competitor probe molecule used per μl of hybridization solution. The volume of hybridization solution used should be between 8 μl and 100 ml, in a particularly preferred embodiment of the method of present invention it is 30 μl.

The hybridization usually lasts between 10 minutes and 12 hours, preferably the hybridization lasts for about 1.5 hours. The hybridization temperature is preferably between 44°C and 48°C, particularly preferably 46°C, whereby the parameter of the hybridization temperature as well as the concentration of salts and detergents in the hybridization solution may be optimized depending on the nucleic acid probes, especially their lengths and the degree to which they are complementary to the target sequence in the cell to be detected. The one skilled in the art is familiar with appropriate calculations.

After hybridization the non-hybridized and excess nucleic acid probe molecules should be removed or washed off, which is usually achieved by a conventional washing solution. This washing solution may, if desired, contain 0.001-0.1%, preferably 0.005-0.05%, particularly preferably 0.01% of a detergent such as SDS, as well as Tris-HCl in a concentration of 0.001-0.1 mol/l, preferably 0.01-0.05 mol/l, particularly preferably 0.02 mol/l, wherein the pH value of Tris-HCl is within the range of 6.0 to 9.0, preferably of 7.0 to 8.0, particularly preferably 8.0. A detergent may be contained, although this is not obligatorily necessary. Furthermore, the washing solution usually contains NaCl, whereby the concentration is 0.003 mol/l to 0.9 mol/l, preferably 0.01 mol/l to 0.9 mol/l, depending on the stringency required. Moreover, the washing solution may contain EDTA, whereby the concentration is preferably 0.005 mol/l. The washing solution may further contain suitable amounts of preservatives known to the expert.

In general, buffer solutions are used in the washing step which can in principle be very similar to the hybridization buffer (buffered sodium chloride solution), except that the washing step is usually performed in a buffer with a lower salt concentration and at a higher temperature, respectively. For theoretical estimation of the hybridization conditions, the following formula may be used:

$$Td = 81.5 + 16.6 \lg[Na^+] + 0.4 \times (\% GC) - 820/n - 0.5 X (\%FA)$$

Td = dissociation temperature in °C

$[Na^+]$ = molarity of the sodium ions

% GC = percentage of guanine and cytosine nucleotides relative to the total number of bases

n = length of the hybrid

% FA = formamide content

Using this formula, the formamide content (which should be as low as possible due to the toxicity of the formamide) of the washing buffer may for example be replaced by a correspondingly lower sodium chloride content. However, the person skilled in the art is, from the extensive literature concerning in situ hybridization methods, aware of the fact that, and in which way, the mentioned contents can be varied. Concerning the stringency of the hybridization conditions, the same applies as outlined above for the hybridization buffer.

The "washing off" of the non-bound nucleic acid probe molecules is usually performed at a temperature in the range of 44°C to 52°C, preferably of 44°C to 50°C and particularly preferably at 46°C for 10 to 40 minutes, preferably for 15 minutes.

The specifically hybridized nucleic acid probe molecules can then be detected in the respective cells, provided that the nucleic acid probe molecule is detectable, e.g., by linking the nucleic acid probe molecule to a marker by covalent binding. As

detectable markers, for example, fluorescent groups, such as for example CY2 (available from Amersham Life Sciences, Inc., Arlington Heights, USA), CY3 (also available from Amersham Life Sciences), CY5 (also obtainable from Amersham Life Sciences), FITC (Molecular Probes Inc., Eugene, USA), FLUOS (available from Roche Diagnostics GmbH, Mannheim, Germany), TRITC (available from Molecular Probes Inc., Eugene, USA), 6-FAM or FLUOS-PRIME are used, which are well known to the person skilled in the art. Also chemical markers, radioactive markers or enzymatic markers, such as horseradish peroxidase, acid phosphatase, alkaline phosphatase, peroxidase may be used. For each of these enzymes a number of chromogens is known which may be converted instead of the natural substrate and may be transformed into either coloured or fluorescent products. Examples of such chromogens are listed in the following table:

Table

Enzyme	Chromogen
1. Alkaline phosphatase and acid phosphatase	4-methylumbelliferyl phosphate (*), bis(4-methylumbelliferyl phosphate, (*) 3-O-methylfluorescein, flavone-3-diphosphate triammonium salt (*), p-nitrophenylphosphate disodium salt
2. Peroxidase	tyramine hydrochloride (*), 3-(p-hydroxyphenyl)-propionate (*), p-hydroxyphenethyl alcohol (*), 2,2'-azino-di-3-ethylbenzothiazoline sulfonic acid (ABTS), ortho-phenylendiamine dihydrochloride, o-dianisidine, 5-aminosalicylic acid, p-ucresol (*), 3,3'-dimethyloxy benzidine, 3-methyl-2-benzothiazoline hydrazone, tetramethylbenzidine

3. Horseradish peroxidase	H_2O_2 + diammonium benzidine
	H_2O_2 + tetramethylbenzidine
4. ß-D-galactosidase	o-nitrophenyl-ß-D-galactopyranoside, 4-methylumbelliferyl-ß-D-galactoside
5. Glucose oxidase	ABTS, glucose and thiazolyl blue

* fluorescence

Finally, it is possible to design the nucleic acid probe molecules in such a way that another nucleic acid sequence suitable for hybridization is present at their 5' or 3' ends. This nucleic acid sequence in turn comprises about 15 to 100, preferably 15-50 nucleotides. This second nucleic acid region may in turn be detected by a nucleic acid probe molecule which is detectable by one of the above-mentioned agents.

Another possibility is the coupling of the detectable nucleic acid probe molecules to a haptene which may subsequently be brought into contact with an antibody recognising the haptene. Digoxigenin may be mentioned as an example of such a haptene. Other examples in addition to those mentioned are well known to the one skilled in the art.

The final evaluation is, depending on the kind of labelling of the probe used, possible, among others, with an optical microscope, epifluorescence microscope, chemoluminometer, fluorometer.

An important advantage of the methods described in this application for the specific detection of drink-spoiling yeasts of the genera *Zygosaccharomyces, Hanseniaspora, Candida, Brettanomyces, Dekkera, Pichia, Saccharomyces* and *Saccharomycodes*, in particular of the species *Zygosaccharomyces bailii*, *Z. mellis*, *Z. rouxii*, *Z. bisporus*, *Z. fermentati*, *Z. microellipsoides*, *Hanseniaspora uvarum*, *Candida intermedia*, *C. crusei (Issatchenkia orientalis)*, *C. parapsilosis*, *Brettanomyces bruxellensis*, *B.*

naardenensis, *Dekkera anomala*, *Pichia membranaefaciens*, *P. minuta*, *P. anomala*, *Saccharomyces exiguus*, *S. cerevisiae*, *Saccharomycodes ludwigii* or for the specific detection of drink-spoiling molds of the genera *Mucor, Byssochlamys, Neosartorya, Aspergillus* and *Talaromyces*, in particular of species *Mucor racemosus*, *Byssochlamys nivea*, *Neosartorya fischeri*, *Aspergillus fumigatus and A. fischeri*, *Talaromyces flavus*, *T. bacillisporus* and *T. flavus,* or for the specific detection of drink-spoiling bacteria of the genera Lactobacillus, Leuconostoc, Oenococcus, Weissella, Lactococcus, Acetobacter, Gluconobacter, Gluconoacetobacter, Bacillus und Alicyclobacillus, in particular of the species *Lactobacillus collinoides*, *Leuconostoc mesenteroides*, *L. pseudomesenteroides*, *Oenococcus oeni*, *Bacillus coagulans*, *Alicyclobacillus ssp.*, *A. acidoterrestris*, *A. cycloheptanicus* and *A. herbarius* compared to the detection methods described above is the exceptional speed. In comparison to conventional cultivation methods which need up to 10 days, the result is obtained within 24 to 48 hours when the methods according to the invention are used.

Another advantage is the ability to perform an accurate differentiation of the drink-spoiling microorganims to be detected. With the methods common up to now no differentiation of the microorganisms was carried out until the genus or species level, as the differentiation was either not possible at all or was too time-consuming.

Another advantage is the specificity of these methods. With the nucleic acid probe molecules used, drink-spoiling yeasts of the genera *Zygosaccharomyces, Hanseniaspora, Candida, Brettanomyces, Dekkera, Pichia, Saccharomyces* and *Saccharomycodes,* in particular the species *Zygosaccharomyces bailii*, *Z. mellis*, *Z. rouxii*, *Z. bisporus*, *Z. fermentati, Z. microellipsoides*, *Hanseniaspora uvarum*, *Candida intermedia*, *C. crusei (Issatchenkia orientalis)*, *C. parapsilosis*, *Brettanomyces bruxellensis*, *B. naardensis*, *Dekkera anomala*, *Pichia membranaefaciens*, *P. minuta*, *P. anomala*, *Saccharomyces exiguus*, *S. cerevisiae*, *Saccharomycodes ludwigii* or drink-spoiling molds of the genera *Mucor,*

Byssochlamys, Neosartorya, Aspergillus and Talaromyces, in particular of the species *Mucor racemosus*, *Byssochlamys nivea*, *Neosartorya fischeri*, *Aspergillus fumigatus* and *A. fischeri*, *Talaromyces flavus*, *T. bacillisporus* and *T. flavus* or drink-spoiling bacteria of the genera *Lactobacillus, Leuconostoc, Oenococcus, Weissella, Lactococcus, Acetobacter, Gluconobacter, Gluconoacetobacter, Bacillus* and *Alicyclobacillus*, in particular of the species *Lactobacillus collinoides*, *Leuconostoc mesenteroides*, *L. pseudomesenteroides*, *Oenococcus oeni*, *Bacillus coagulans*, *Alicyclobacillus ssp.*, *A. acidoterrestris*, *A. cycloheptanicus* and *A. herbarius* can be detected in a highly specific manner. By the visualisation of the microorganisms a visual control may be performed at the same time. False-positive results, such as often occurring in polymerase chain reaction, are therefore ruled out.

Another advantage of the methods according to the invention is their ease of use. Thus, using this methods, large numbers of samples can be easily tested regarding the presence of the mentioned microorganims.

Finally, an important advantage compared to the state of the art is the possible simultaneous detection of several of the mentioned microorganisms by the use of respective mixtures of probes. Following this approach all practise relevant drink-spoiling microorganisms can be detected in a few tests.

Different probes may hereby be coupled with different labels, so that the various, detected micororganisms may be discriminated in an easy and reliable way. For example, a first oligonucleotide may be specifically labelled with a green fluorescence dye and serves for the detection of a certain genus or species of microorganism. A second oligonucleotide is also specifically labelled with, for instance, a red fluorescence dye and serves for the detection of a second genus or species of microorganism. The oligonucleotides referred to as competitor probes remain non-labelled and prevent the binding of the first and/or the second oligonucleotide probe to bacteria which do not belong to the genera or species to be

detected. The different labels, e.g. the green fluorescence dye on the one hand and the red fluorescence dye on the other hand may be differentiated in an easy manner, for example by using different filters in fluorescence microscopy.

The methods according to the invention may be used in various ways.

For example, non-alcoholic drinks (e.g. fruit juices, fruct nectars, fruit concentrates, mashed fruits, soft drinks and waters) may be tested for the presence of the microorganisms to be detected.

For example, also environmental samples can be tested for the presence of the micororganisms to be detected. Theses samples may be, for example, collected from soil or be parts of plants.

The method according to the invention may further be used for testing sewage samples or silage samples.

The method according to the invention may further be used for testing medicinal samples, e.g. stool samples, blood cultures, sputum, tissue samples (also sections), wound material, urine, samples from the respiratory tract, implants and catheter surfaces.

Another field of use of the method according to the invention is the control of food. In preferred embodiments the food samples are obtained from milk or milk products (yogurt, cheese, curd, butter, buttermilk), drinking water, alcoholic drinks (beer, wine, spirits), bakery products or meat products.

A further field of use of the method according to the invention is the analysis of pharmaceutical and cosmetic products, e.g. ointments, creams, tinctures, juices, solutions, drops, etc.

Furthermore, according to the invention, kits for performing the respective methods are provided. The hybridization arrangement contained in these kits is described for example in German patent application 100 61 655.0. Express reference is herewith made to the disclosure contained in this document with respect to the in situ hybridization arrangement.

Besides the described hybridization arrangement (referred to as VIT reactor), the most important component of the kits is the respective hybridization solution (referred to as VIT solution) with the nucleic acid probe molecules specific for the microorganisms to be detected, which are described above (VIT solution). Further contained are the respective hybridization buffer (Solution C) and a concentrate of the respective washing solution (Solution D). Also contained are optionally fixation solutions (Solution A and Solution B) as well as optionally an embedding solution (finisher). Optionally, solutions are contained for performing in parallel a positive control as well as of a negative control.

The following example is intended to illustrate the invention without limitation.

Example

Specific rapid detection of drink-spoiling microorganisms in a sample

A sample is cultivated for 20 to 48 hours in a suitable manner. For the detection of yeasts and molds cultivation may be performed, for example, in SSL-bouillon for 24 hours at 25°C. For the detection of lactic acid bacteria the cultivation may be performed for example in MRS-bouillon for 48 hours at 30°C. For the detection of aceteic acid bacteria the cultivation may be performed, for example, on DSM-agar for 48 hours at 28°C. For the detection of bacilli, in particular *B. coagulans,* the cultivation may be performed, for example, on dextrose-casein-peptone-agar for 48

hours at 55°C. For the detection of alicyclobacilli, the cultivation may be performed, for example, in BAM-bouillon for 48 hours at 44°C.

To an aliquot of the culture the same volume of fixation solution (Solution B, ethanol absolute) is added. Alternatively, an aliquot of the culture may be centrifuged (4000 g, 5 min, room temperature) and, after discarding the supernatant, the pellet may be dissolved in 4 drops of fixation solution (Solution B).

For performing the hybridization a suitable aliquot of the fixed cells (preferably 5 µl) is applied onto a slide and dried (46°C, 30 min, or until completely dry). Alternatively, the cells may also be applied to other carrier materials (e.g. a microtiter plate or a filter). The dried cells are then completely dehydrated by again adding the fixation solution (Solution B). The slide is again dried (room temperature, 3 min, or until completely dry).

Then the hybridization solution (VIT solution, hybridization buffer containing labeled probe molecules) containing the above described nucleic acid probe molecules specific for the microorganisms to be detected, is applied to the fixed, dehydrated cells. The preferred volume is 40 µl. The slide is then incubated (46°C, 90 min) in a chamber humidified with hybridization buffer (Solution C), preferably the VIT reactor (c. f. DE 100 61 655.0).

Then the slide is removed from the chamber, the chamber is filled with washing solution (Solution D, diluted 1:10 with distilled water) and the slide is incubated in the chamber (46°C, 15 min).

Then the chamber is filled with distilled water, the slide is briefly immersed and then air-dried in lateral position (46°C, 30 min or until completely dry).

Then the slide is embedded in a suitable medium (Finisher).

Finally, the sample is analyzed with the help of a fluorescence microscope.